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Liposomal drug formulations in cancer therapy: 15 years along the road

Marije Slingerland[1], Henk-Jan Guchelaar[2] and Hans Gelderblom[1]

[1] Department of Medical Oncology, Leiden University Medical Center, The Netherlands
[2] Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, PO Box 9600, 2300 RC, Leiden, The Netherlands

Liposomes as pharmaceutical drug carriers were developed to increase antitumour efficacy and decrease drug toxicity. Doxorubicin HCl liposomal injection was the first liposomal encapsulated anticancer drug to receive clinical approval. To date, virtually all traditional anticancer drugs have been encapsulated in liposomes.

The majority of clinical studies only support the concept of a decreased toxicity and better tolerability of the liposomal anticancer drug. Although liposomal anticancer drugs have grown to maturity in several indications and are now in widespread further development programmes using their theoretical advantages to fulfil the high expectations, further studies are warranted – including the development of novel liposomal formulations.

Introduction

Many current anticancer drugs have non-ideal pharmaceutical and pharmacological properties such as low aqueous solubility, irritant properties, lack of stability, rapid metabolism, unfavourable pharmacokinetics and non-selective drug distribution, which can lead to a number of adverse consequences, including lack of or suboptimal therapeutic activity, dose-limiting side effects and poor patient quality of life [1]. From the drug delivery perspective, this might not only result in low bioavailability of the anticancer drug at the site of action (i.e. inside the cancer cells) but also in high organ toxicity that limits the maximal tolerable dose. Nanoscale drug delivery systems, defined as drug delivery systems having particle diameters of approximately 100 nm or less, are attracting considerable attention as a means of overcoming some of the limitations of conventional anticancer drug therapy. Liposomes and other lipid-based drug delivery systems are the archetypal nanoscale drug delivery systems. The first product, liposomal amphotericin B (Ambisome®), which is indicated for fungal infections, received clinical approval in 1990. Liposomes are simple, self-assembling systems that consist of a bilayer membrane surrounding an aqueous interior compartment. They are generally formed from naturally occurring phospholipids and cholesterol, rendering them readily biodegradable (Fig. 1, [2]). Considerable flexibility is possible in the design of liposomes with regard to, for example, their composition, size and drug release characteristics. Liposomal nanoparticles are designed to be multifunctional, with different components providing control over such properties as elimination half lives, permeability, biodistribution and targeting specificity [1].

Doxorubicin HCl liposomal injection (Caelyx® in Europe, Doxil® in the USA), which received marketing approval in 1995, was the first nanoscale delivery system to receive clinical approval in cancer therapy for acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma [3]. Currently, virtually all traditional anticancer drugs have been encapsulated in liposomes using different technologies and many of them have entered clinical trials as cancer-imaging agents and/or anticancer therapeutics, indicating that this is a rapidly developing field that justifies review.

Here, we focus on the liposomal anticancer drugs that are available in the clinic, including discussion on the specific adverse effects of liposomes. We start with a short description of the principles of liposomal delivery.

Corresponding author:. Guchelaar, H.-J. (h.j.guchelaar@lumc.nl)

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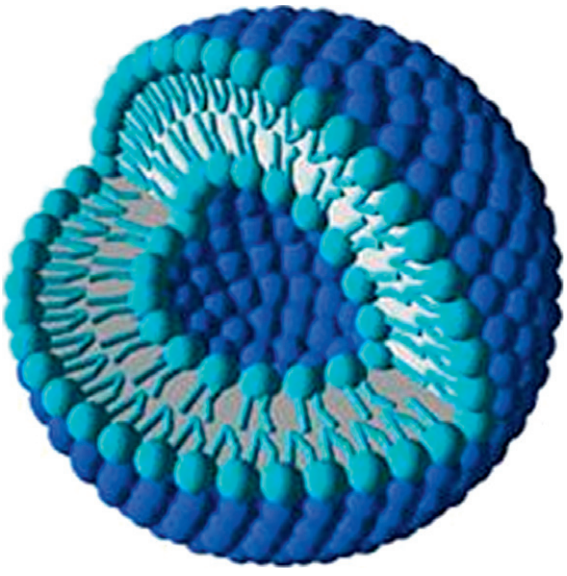


FIGURE 1
Liposome.

Principles of liposomal drug delivery

Theoretically, liposomes have a couple of advantages over non-capsulated drugs [4], first of which is their improved pharmacokinetics and drug release. In 2010, in a meta-analysis, Sidone *et al*. demonstrated that the pharmacokinetic (PK) variability of liposomal agents is 2.7 fold or 16.7 fold greater than non-liposomal agents, measured by ratio of the coefficient of variation (CV) to AUC, AUC CV%, and ratio of AUC_{max} to AUC_{min}, respectively [5]. A second advantage of liposomal drugs is their enhanced cellular penetration, for which exist different mechanisms, such as fusion of the liposomal membrane with the cellular plasma membrane [4].

A third advantage is the possibility of selectively targeting anticancer drugs to the tumour, preventing the side effects of drugs related to effects in healthy tissues and enhancing the uptake of the drug by the targeted cells [4]. The fourth theoretical advantage of liposomal drugs is the ability to include several active ingredients in one complex liposomal drug delivery system. Clinical evidence supports the hypothesis of Goldie and Coldman: that treating cancers with all the available effective agents simultaneously provides the greatest chance of eliciting a cure [6]. Combination chemotherapy carried out with synergistic drugs is considered as a basis for improving its effectiveness. The ultimate goal of research is to prepare a product that encompasses traditional cytotoxic agents and new molecularly targeted modalities with optimum therapeutic effects and acceptable toxicity for healthy tissues, although this is difficult to achieve [6].

Clinical use of liposomal drugs

At present, several liposomal anticancer drugs are available in the clinic (Table 1) or are in advanced stages of clinical development (Table 2). Approved drugs include pegylated liposomal doxorubicin (Doxil®/Caelyx®), nonpegylated liposomal doxorubicin (Myocet®), liposomal daunorubicin (DaunoXome®) and liposomal cytarabine (DepoCyte®).

We searched the literature (Pubmed) on this topic using a combination of the medical subject heading (MeSH) terms 'antineoplastic agents', 'daunorubicin', 'cytarabine', 'cisplatin' and 'clinical trials Phase III', as well as search terms 'pegylated liposomal doxorubicin', 'chemotherapy', 'anticancer', 'antineoplastic', 'liposomal', 'liposomic', 'liposomes' and 'liposome', on 3 September 2011.

TABLE 1
Overview of approved liposomal anticancer drugs

Available liposomal anticancer drug	Indication (for exact indication see text)	Phase III study	Refs
Nonpegylated liposomal doxorubicin	AIDS-related Kaposi's sarcoma	Stewart *et al.* 1998	[8]
		Northfelt *et al.* 1998	[9]
		Cianfrocca *et al.* 2010	[10]
	Metastatic ovarian cancer	Gordon *et al.* 2001	[11]
		Pignata *et al.* 2009	[15]
		Markman *et al.* 2010	[16]
		Pujade-Lauraine *et al.* 2010	[17]
	Metastatic breast cancer	Keller *et al.* 2004	[18]
		Chan *et al.* 2004	[20]
		Sparano *et al.* 2009	[21]
		Alba *et al.* 2010	[22]
	Multiple myeloma	Rifkin *et al.* 2006	[24]
		Orlowski *et al.* 2007	[26]
		Sonneveld *et al.* 2008	[25]
Liposomal daunorubicin	AIDS-related Kaposi's sarcoma	Gill *et al.* 1996	[7]
	Acute myeloid leukaemia	Latagliata *et al.* 2008	[27]
Liposomal cytarabine	Lymphomas or leukaemia with meningeal spread	Glantz *et al.* 1999	[28]

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TABLE 2

Some liposomal chemotherapeutic anticancer drugs at various stages of development

Drug	Encapsulated chemotherapeutic agent	Development stage	Refs
ThermoDox®	Doxorubicin	Phase II	[45]
JNS002	Doxorubicin	Phase II	[41]
Liposomal annamycin	Annamycin	Phase II	[46]
LEM	Mitoxantrone	Preclinical	[47]
SPI-77	Cisplatin	Phase II	[48–51]
Lipoplatin	Cisplatin	Phase III	[52]
LiPlaCis	Cisplatin	Phase I	[53]
L-NDDP/aroplatin	Cisplatin analogue	Phase II	[54,55]
MBP426	Oxaliplatin	Phase I	[56]
NL CPT-11	Nanoliposomal camptothecine	Trial	http://www.clinicaltrials.gov/
L9NC	9-nitro-20 (S)-camptothecin	Trial	http://www.clinicaltrials.gov/
IHL-305	Irinotecan	Phase I	[57]
LE-SN38	SN38 (active metabolite of irinotecan)	Trial	http://www.clinicaltrials.gov/
PEP02	Irinotecan	Phase I	[58]
OSI211	Lurtotecan	Phase II	[59,60]
TLI	Topotecan	Trial	http://www.clinicaltrials.gov/
PNU-93914	Paclitaxel	Trial	http://www.clinicaltrials.gov/
LEP-ETU	Paclitaxel	Trial	http://www.clinicaltrials.gov/
Marqibo®	Vincristine	Phase II	[61]
VLI	Vinorelbine	Trial	http://www.clinicaltrials.gov/
CPX-1	Fixed combination of irinotecan and floxuridine	Phase I	[62]
CPX-351	Fixed combination of cytarabine and daunorubicin	Phase I	[63]

Liposomal formulations of anthracyclines are being used today for the treatment of HIV-associated Kaposi's sarcoma, ovarian cancer and breast cancer.

HIV-associated Kaposi's sarcoma

In the 1990s there were already positive reports of liposomal formulations of anthracyclines with high response rates in the treatment of AIDS-related Kaposi's sarcoma. In 1996, Gill *et al.* convincingly showed that a nonpegylated liposomal formulation of daunorubicin 40 mg/m^2 given every two weeks had considerably less toxicity than the doxorubicin, bleomycin and vincristine regimen without compromising efficacy. The overall response rate was 25% versus 28% [7]. In 1998 Stewart *et al.* reported on a multicentre Phase III study that compared pegylated liposomal doxorubicin with the combination of bleomycin and vincristine and showed that the liposomal product is an effective treatment for AIDS-related Kaposi's sarcoma with a higher overall response rate (58.7% versus 23.3%, $P < 0.001$) than the bleomycin and vincristine combination. They reported that it was well tolerated but more myelosuppressive [8]. In 1998 Northfelt *et al.* reported on a Phase III study that compared pegylated liposomal doxorubicin 20 mg/m^2 given every two weeks with doxorubicin, bleomycin and vincristine, during which patients that received pegylated liposomal doxorubicin experienced less toxicity and a higher overall response rate (45.9% versus 24.8%, $P < 0.001$) [9]. In 2010 Cianfrocca *et al.* demonstrated in a Phase III study that treatment with either paclitaxel or pegylated liposomal doxorubicin appears to produce significant improvements in pain and swelling in patients with advanced, symptomatic, HIV-associated Kaposi's sarcoma treated in the highly active antiretroviral therapy (HAART) era. Comparing the paclitaxel and pegylated liposomal doxorubicin results revealed similar overall response rates (56% versus 46%, $P = 0.49$) [10].

Ovarian carcinoma

In 2001, a Phase III study in patients with epithelial ovarian carcinoma that had recurred after, or was not responsive to, first-line platinum-based chemotherapy was published by Gordon *et al.* to compare the efficacy and safety of pegylated liposomal doxorubicin and topotecan. They concluded that the comparable efficacy (overall response rates: 19.7% versus 17.0%, $P = 0.390$), favourable safety profile and convenient dosing support the role of pegylated liposomal doxorubicin as a valuable treatment option in this patient population [11]. Based on Phase II results [12–14] and efficacy data from this Phase III study, Caelyx® received FDA approval in June 1999 for the treatment of metastatic carcinoma of the ovary in patients with disease that is refractory to paclitaxel- and platinum-based chemotherapy regimens. Since the approval, much research has been done on liposomal doxorubicin. In 2009, based on the efficacy of pegylated liposomal doxorubicin in relapsed ovarian cancer, Pignata *et al.* demonstrated in a Phase III study that pegylated liposomal doxorubicin plus carboplatin also has activity as a first-line treatment for advanced ovarian cancer (overall response rate of 68%, which exceeded the minimum required for study continuation) [15]. In 2010 Markman *et al.* demonstrated in their Phase III study that carboplatin plus

pegylated liposomal doxorubicin in recurrent ovarian cancer had a favourable impact on progression-free survival (12 versus 8 months, $P = 0.02$), although the effect on overall survival was not statistically significant (median: 31 versus 18 months, $P = 0.2$) [16]. In 2010 Pujade-Lauraine *et al.* published a randomized, multicentre, Phase III noninferiority trial that demonstrated superiority in progression-free survival (11.3 versus 9.4 months, $P = 0.005$), and a better therapeutic index of pegylated liposomal doxorubicin with carboplatin over standard carboplatin and paclitaxel [17].

Breast cancer

Also, in patients with metastatic breast cancer liposomal doxorubicin seemed to be effective. In 2004, Keller *et al.* published a randomized Phase III trial to compare the efficacy of pegylated liposomal doxorubicin with that of a common salvage regimen in patients with taxane-refractory advanced breast cancer. Patients in the control group received either vinorelbine or mitomycin C plus vinblastine, regimens previously shown to have moderate efficacy (median overall survival: 10.4 months versus 9.0 months, $P = 0.57$). They concluded that pegylated liposomal doxorubicin has efficacy comparable to that of common salvage regimens in patients with taxane-refractory metastatic breast cancer, thereby representing a useful therapeutic option [18]. The same year, O'Brien *et al.* published a Phase III trial to demonstrate that efficacy of pegylated liposomal doxorubicin is comparable to doxorubicin [progression free survival 6.9 versus 7.8 months, hazard ratio (HR) = 1.00], with significantly reduced cardiotoxicity (HR = 3.16, $P < 0.001$), myelosuppression, vomiting and alopecia in first-line treatment of women with metastatic breast cancer [19]. Also in 2004 Chan *et al.* showed that liposomal doxorubicin is an acceptable alternative to epirubicin as a first-line treatment for patients with metastatic breast cancer (overall response rates: 46% and 39%, $P = 0.42$) [20]. In 2009 Sparano *et al.* demonstated that pegylated liposomal doxorubicin was more effective than docetaxel alone in women with metastatic breast cancer who experienced relapse at least 1 year after prior adjuvant anthracycline therapy (median time to progression: 7.0–9.8 months, $P = 0.000001$; and the overall response rate from 26% to 35%, $P = 0.0085$), although overall survival was similar among the two groups (HR = 1.02, 95% CI, 0.86–1.22). This was without an increase in cardiac toxicity, although mucocutaneous toxicity was more common [21]. In 2010 Alba *et al.* demonstrated in their Phase III study that maintenance chemotherapy with pegylated liposomal doxorubicin is well tolerated and offers improved time to progression of 3.3 months (8.4 versus 5.1 months, $P = 0.0002$) in patients with metastatic breast cancer following first-line chemotherapy [22].

Haematological malignancies

For a few years liposomal anthracyclines have also been tested in the treatment of haematological malignancies.

In 2003 Dimopoulos *et al.* reported a multicentre trial that indicated that vincristine, doxorubicin and dexamethasone bolus and vincristine, liposomal doxorubicin and dexamethasone can be administered to outpatients and can provide an equal opportunity of rapid response in many patients with multiple myeloma (overall response of 61.4% and 61.3%) [23]. In 2006 Rifkin *et al.* published a Phase III trial to show that pegylated liposomal doxorubicin, vincristine and dexamethasone provide similar efficacy (objective response rates: 44% versus 41% progression-free survival, $P = 0.69$; and overall survival, $P = 0.67$) with significant reduction in toxicity with doxorubicin, vincristine and dexamethasone in patients with newly diagnosed multiple myeloma. Notwithstanding these promising results, the authors concluded that the optimal management of patients with newly diagnosed myeloma still requires further study [24]. Sonneveld *et al.* showed in 2008 that pegylated liposomal doxorubicin plus bortezomib significantly prolonged time to progression compared with bortezomib alone (270 days versus 205 days) in patients with recurrent or refractory multiplemyeloma who received prior thalidomide/lenalidomide therapy [25]. A year earlier, in the same Phase III study, Orlowski *et al.* showed that pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone improved time to progression (6.5 months versus 9.3 months) in relapsed or refractory multiple myeloma [26].

Recently, Latagliata *et al.* explored the efficacy of liposomal daunorubicin versus daunorubicin in acute myeloid leukaemia patients aged older than sixty years. Liposomal daunorubicin seemed to improve overall survival and disease-free survival in the long-term follow-up, because of a reduction on late relapses (59% versus 78% at 24 months, $P = 0.064$). The authors concluded that liposomal daunorubicin could have a possible beneficial role in acute myeloid leukaemia treatment although further testing would be useful [27].

Liposomal cytarabine is approved for the treatment of lymphomas with meningeal spread and is the only liposomal drug administered for intrathecal administration. Although liposomal cytarabine is increasingly used for the treatment (and prophylaxis) of central nervous system involvement in patients with leukaemia or lymphoma, many of the recently presented clinical trials on liposomal cytarabine were retrospective in nature or used this drug on a compassionate use basis. So far, one randomized Phase III study has shown significantly better response rates in patients with lymphomatous meningitis who received liposomal cytarabine compared with cytarabine. The authors of this randomized trial concluded that liposomal cytarabine injected once every two weeks produced a high response rate (71% versus 15%, $P = 0.006$) and a better quality of life as measured by Karnofsky score ($P = 0.041$) relative to that upon treatment with free cytarabine injected twice a week [28].

Epithelial malignancies

Liposomal cisplatin was developed for the treatment of epithelial malignancies. Initial safety and response results of a randomized Phase III study with liposomal cisplatin in the treatment of advanced squamous cell carcinoma of the head and neck showed that liposomal cisplatin seems to reduce the renal and haematological toxicity, as compared with conventional cisplatin, to a clinically relevant extent. This reduction of side effects will influence the chance to preserve the dose-density of chemotherapy and, thereby, the efficacy of treatment. The efficacy results showed 38.8% objective partial remission in the cisplatin arm of the trial versus 19% in the lipoplatin arm. However, 64% of the patients achieved stable disease while being treated with lipoplatin/5-fluorouracil (5-FU), versus 50% in the cisplatin/5-FU arm [29]. In 2010, Stathopoulos *et al.* showed in a Phase III study that

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liposomal cisplatin in combination with paclitaxel was much less toxic than the cisplatin in combination with paclitaxel, whereas time to tumour progression (6.5 versus 6 months) and survival (9 versus 10 months) were similar in chemotherapy-naive patients with inoperable non-small-cell lung cancer [30].

The majority of clinical studies we have described are only supporting the concept of a decreased toxicity and better tolerability of the liposomal anticancer drug, there is a lack of available information regarding the greater clinical antitumour activity. None of the studies showed a better overall survival for the liposomal drug when directly compared to the non-liposomal variant. One of the reasons for this could be the inefficient drug release from the liposomes, as described by Seynhaeve *et al*. in 2007, showing that intact Doxil® liposomes could be visualised within living tumour cells [31].

Because no direct comparative data are available on the efficacy of the drugs further studies with novel liposome encapsulated anticancer drugs are warranted to provide conclusive evidence for increased efficacy.

Also, no direct comparative data are available on the tumour distribution of drugs by administering the same doses given as a free drug or incapsulated in liposomes. As far as the distribution is concerned, one should perform studies giving the free drug and the liposomal formulation at the same time – labelling the drug in different ways and thus having interpretable results on the difference of distribution according to the method of administration.

Liposome-specific adverse effects

Although almost all studies show that liposomal formulations of anticancer drugs are less toxic than the non-encapsulated formulations, some liposome-specific adverse effects such as various skin reactions, and also hypersensitivity reactions, were reported.

Skin reactions

In 2000, Lotem *et al*. reported a study to show skin toxic effects of polyethylene-glycol-coated liposomal doxorubicin. In 60 patients four patterns of skin eruptions were seen: (i) hand-foot syndrome; (ii) diffuse follicular rash; (iii) intertrigo-like eruptions; and (iv) new formation of melanotic macules. The most common effect was the hand-foot syndrome, which was more pronounced, frequent and disabling with short dose intervals. This side effect is not a side effect of doxorubicin itself. Compared with doxorubicin, liposomal doxorubicin has a long elimination half life and is highly stable, thus providing a slow release pool of drug to tumour and other tissues. It preferably localises in the skin and deposits a substantial fraction of the administered drug locally. Inflamed skin is especially susceptible to liposome localisation. The palms, soles and areas of repeated friction or trauma apparently achieve increased concentrations of liposomal doxorubicin as a result of the rich capillary network at their thickened papillary dermis and increased blood flow [32].

Hypersensitivity reactions

Chan *et al*. described an episode of hypersensitivity reaction associated with the infusion of liposomal doxorubicin in an ovarian cancer patient during her first cycle of chemotherapy [33]. Hypersensitivity or infusion reactions with (pegylated) liposomes are well known and yet poorly understood. This type of hypersensitivity reaction is an acute transient malaise that develops in patients within minutes of vesicle infusion and is typically observed only during the first cycle of exposure. The haemodynamic, respiratory, cutaneous and subjective manifestations include hypotension or hypertension, dyspnea, flushing, rash and feeling of choking. Up to 30.8% of the patients experienced any type of hypersensitivity reaction [30,34–42]. Although in practice severe hypersensitivity reactions to liposomal formulations are very uncommon. Unlike most chemotherapy, induced hypersensitivity reactions are IgE-mediated and the mechanism of liposomal reaction is described as a type I hypersensitivity reaction related to complement activation [43]. Slowing of the rate, or stopping the infusion, along with standard measures of anaphylaxis prevention and treatment (e.g. antihistamines, corticosteroids, epinephrine, bronchodilators or supportive therapy with fluids) usually seem to be sufficiently effective. However, considering that cardiopulmonary distress is a major physiological consequence that can lead to cardiac anaphylaxis, the prediction and prevention of this reaction seems to be crucial in patients with cardiovascular abnormalities. Liposome reactions in such patients can be life threatening, despite all treatment and preventive measures [44].

Concluding remarks

In recent years, liposomes as pharmaceutical drug carriers have received considerable and increasing attention. Several Phase II and III studies have shown increased antitumour efficacy and decreased toxicity and also several liposomal anticancer drugs are already available in the clinic for Kaposi's sarcoma, ovarian cancer and breast cancer. Further studies with liposome-encapsulated anticancer drugs, including the development of novel liposomal formulations, are warranted to provide evidence for increased efficacy and tolerability as compared with their non-liposomal counterparts. Fifteen years down the road we can conclude that liposomal anticancer drugs have grown to maturity in several indications and are in broad further development using their theoretical advantages to fulfil the high expectations.

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