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Review

Ionic gradient liposomes: Recent advances in the stable entrapment and prolonged released of local anesthetics and anticancer drugs

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ABSTRACT

Liposomes have established themselves as great pharmaceutical carriers over the past three decades. These phospholipid vesicular systems have undergone great technical advances including remote drug loading, targeted delivery, and combinatorial drug therapy. Ionic gradient liposomes (IGL) necessitates active loading of the drug in preformed vesicles exhibiting a transmembrane pH or ion gradient, with a low intra liposome pH (~ 4-5), and a high outside pH (~7-8). It allows high drug encapsulation and prolonged release, particularly for amphipathic weak acids and weak bases. Most local anesthetics (Bupivacaine, Ropivacaine, Tetracaine, and others) have a pka in the range of 7-9, which makes them ideal candidates for their entrapment in IGL. The same is true for most anthracyclines which have great anti-tumor properties (Doxorubicin, Daunorubicin, Idarubicin, and others). Many FDA approved liposomal drugs utilise ion gradient for their encapsulation. Considering their immense utility, we summarize here in this review, the recent contributions made by various research groups utilizing IGL, to accentuate the development of these carriers in drug delivery. This would possibly be helpful in carrying new investigations and further contributions in the optimization and advancements of new drugs for better therapeutics.

1. Introduction

The application of liposomes in drug delivery needs little introduction. These are excellent biocompatible, bio degradable and non-toxic carriers, exhibiting an extended circulation life with enhanced permeation and retention effect, which favors its bio-distribution [1]. They are well known to prolong the release of the entrapped pharmaceutical and decrease their systemic as well as organ toxicity significantly [2]. This is very much evident from the fact that the FDA has approved more than 15 liposomal drugs (Table 1), and is currently in clinical use.

Liposomes comprise of lipid bilayers (one or more) surrounding an aqueous core. Two different environments in these carriers make them highly suitable for their use in the delivery of a broad range of hydrophilic, hydrophobic and amphipathic pharmaceuticals [3]. The nature of the lipid (size, the degree of saturation and class) and the preparation method determines important features such as encapsulation and release of the encapsulated moiety. Phosphatidylcholine (PC) is by far the most widely used lipid in liposome preparations, followed by cholesterol [4] and other phospholipids such as phosphatidyl ethanolamine (PE). The PC includes egg PC (EPC), hydrogenated soy PC (HSPC), 1, 2-di-myristoyl-sn-glycero-3-phosphocholine (DMPC), Dipalmitoylphosphatidylcholine (DPPC), mono stearoyl PC (MSPC) and others. Differences in the properties of various lipids used, play a vital role in imparting specific characters to these carriers, including bilayer fluidity, vesicles size and stability [5] as discussed in details subsequently.

Liposomes are broadly classified as small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multilamellar vesicles (MLV) or large multivesicular vesicles (LMVV), on the basis of size. The size ranging from less than 100 nm for SUV, 100-1000 nm for LUV and more than 1 μm for MLV and LMVV. Also, they differ in the number of lipid

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Table 1
FDA approved liposomal drugs, composition and treatment.

S No	Drug	Composition	Treatment
1	Myocet (Liposomal doxorubicin)	EPC: cholesterol (55:45 molar ratio).	combinational therapy for treatment of recurrent breast cancer
2	Doxil, Caelyx (Liposomal doxorubicin)	HSPC: cholesterol: PEG 2000-DSPE (56:39:5 molar ratio). Loaded by an ammonium sulfate gradient.	refractory Kaposi's sarcoma, recurrent breast cancer and ovarian cancer
3	LipoDox (Liposomal doxorubicin)	DSPC: cholesterol: PEG 2000-DSPE (56:39:5 molar ratio)	refractory Kaposi's sarcoma, recurrent breast cancer and ovarian cancer
4	Thermodox (Liposomal doxorubicin)	DPPC, MSPC and PEG2000-DSPE	primary liver cancer (Hepatocellular carcinoma) and also recurrent chest wall breast cancer
5	DaunoXome (Liposomal Daunorubicin)	DSPC and cholesterol (2:1) molar ratio	Kaposi's sarcoma.
6	Ambisome (Liposomal Amphoteracin B)	HSPC, DSPG, cholesterol and amphoteracin B in 2:0.8:1:0.4 molar ratio.	fungal infection
7	Marqibo (Liposomal vincristine)	egg sphingomylin and cholesterol	Metastatic malignant uveal melanoma.
8	Visudyne (Liposomal verteporfin, PBD-MA)	PBD-MA:EPG:DMPC in 1:05:3:5 molar ratio	Age-related macular degeneration, pathologic myopia and ocular histoplasmosis.
9	DepoCyt (Liposomal cytarabine)	Cholesterol: Triolein: Dioleoylphosphatidylcholine (DOPC): Dipalmitoylphosphatidylglycerol (DPPG) in 11:1:7:1 molar ratio.	Neoplastic meningitis and lymphomatous meningitis.
10	DepoDur (Liposomal morphine sulfate)	Cholesterol: Triolein: DOPC:DPPG in 11:1:7:1 molar ratio	Postoperative pain following major surgery.
11	Arikace (Liposomal amikacin)	DPPC and Cholesterol	Lung infections due to susceptible pathogens.
12	Lipoplatin (Liposomal cisplatin)	DPPG, Soy PC, cholesterol and PEG2000-DSPE	Epithelial malignancies such as lung, head and neck, ovarian, bladder and testicular cancers.
13	LEP-ETU (Liposomal Paclitaxel)	DOPE, cholesterol and cardiolipin	Ovarian, breast and lung cancer (phase III of clinical trials)
14	Epaxal (Hepatitis A vaccine)	DOPC/DOPE in 75:25 molar ratio	vaccine adjuvant
15	Exparel (Liposomal Bupivacaine)	Cholesterol (4.7 mg/ml), DPPG (0.9 mg/ml), tricaprylin (2.0 mg/ml), DEPC (8.2 mg/ml)	Anesthesia

Abbreviations: Egg phospatidyl choline (EPC)hydrogenated soy PC (HSPC), polyethylene glycol 2000- 1,2-distearoyl-sn-glycero-3-phosphoethanolamin(PEG 2000-DSPE), 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-di-myristoyl-sn-glycero-3-phosphocholine (DMPC), Dipalmitoylphosphatidaylcholine (DPPC), mono steroyl PC (MSPC) and 1,2-Distearoyl-sn-glycero-3-phosphoglycerol (DSPG), DOPG, Dioleoylphosphatidylcholine (DOPC), Dipalmitoylphosphatidylglycerol (DPPG), and 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dierucoyl phosphatidylcholine (DEPC).

bilayers. SUV and LUV have a single lipid bilayer enclosing the inner aqueous core. The MLV has multiple concentric lipid bilayers with an aqueous phase between each bilayer while the LMVV comprise of multiple vesicles encapsulated in a large vesicle. The difference in size and pattern is instrumental in creating differences in various aspects of drug behavior. For example, hydrophobic drugs would prefer to be better encapsulated in the MLV, due to the presence of relatively large lipid bilayer area. Hydrophilic drugs would be better entrapped in LUV and mostly in LMVV, owing to its very large aqueous area. The more detailed discussion is included in the upcoming text, with special reference to gradient liposomes.

Liposomes are also classified on the basis of the charge on their surface. These include neutral, cationic (positive surface charge) and anionic (negative surface charge) liposomes. The methods of preparation remain the same except that appropriate amount of positive or negative charged lipid is added during the preparation. Cationic liposomes utilise 1, 2-dioleoyl-snglycero-3-phosphoethanolamine (DOPE) or 1, 2-dioleoyl-3-trimethylammonium (DOTAP), while anionic liposome are made by the addition of anionic lipids such as 1, 2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DSPG), in addition to other lipids. The potential of cationic liposome is extensively being explored for the delivery of DNA [6]. More recently, the anionic liposome has been tested for their diffusion in the mucus layer in the intestine *via* oral administration [7].

1.1. Encapsulation of drugs (in general) in conventional liposomes

Conventional liposomes have been extensively investigated and proved promising as controlled drug deliverers, and also in combating the toxicity of the free drug at similar and higher doses [8]. Liposome-based formulations have been reported to prolong drug release significantly as well as increase their therapeutic index [9,10]. This slow drug release may be the major contributor to the decreased systemic as well as organ (hepatic, renal and others) toxicity [11]. A sustained release from the drug entrapped liposomal formulations ensures the drug availability within the therapeutic band for longer durations, making liposomes such efficient carriers. Currently, various formulations of liposomes have also received great interest and application in areas of immunology, diagnostics, pharmacy and medicine, ecology, cosmetics, cleansing and food industry [12].

1.2. Local anesthetics

Local anesthetics (LA) are of utmost pharmacological significance in suppressing acute and chronic pain. They are membrane destabilizing drugs and act largely by reducing the influx of sodium into the neuronal cell membrane, blocking the sodium specific ion channels, finally inhibiting a signal conduction and hence propagation of nerve impulse. Most of the clinically used LAs belong to one of the two classes namely aminoester and aminoamide (Fig. 1). The first group includes Articaine, Bupivacaine (BVC), Cinchocaine (CIN)/Dibucaine (DBC), Etidocaine (EDC), Levobupivacaine, Lidocaine (LDC), Mepivacaine, Prilocaine (PLC), Ropivacaine (RVC) and Trimecaine while the second includes Tetracaine (TTC), Dimethocaine, Piperocaine, Procaine, Chloroprocaine, Propoxycaine, Cyclomethycaine, and Cocaine [13].

In spite of their immense pharmacological potency, these are low molecular weight compounds which rapidly get metabolized, restricting the duration of anesthesia [14]. One such drug is RVC which provides only 2–4 h of effective anesthesia [15]. Besides, systemic toxicity is also a limitation in the application of LA. Several approaches are now being used to extend the anesthetic effect of the drug. Association of various LAs with carriers including different kinds of polymers, cyclodextrins and lipid complexes has enabled slow and sustained release for longer durations [16]. Benzocaine (BZC), widely used as a topical anesthetic, however, is an ester-type LA. It lacks the terminal amino group typical of most LAs and does not ionize over a large range