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Liposomes encapsulating cyclodextrin enclosed hydrophobic anti-cancer drugs: A novel drug delivery system for cancer

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ABSTRACT

In the current situation in the health care industry is to face many challenges to cure the cancer. The second highest deaths were noticed by the cancer in society. Therefore, the efficient treatment is required. There many treatmentsare available to cure the cancer but not found much efficient. The advance development in the hydrophobic drug in the form of water-soluble drug cyclodextrin complex in liposomes has been investigated. This new investigation in to the complex in liposomes play key role in combination of the cyclodextrin and liposomes in the one common system. Such system would potentially increase the drug to lipid mass ratio. In comparison to conventional treatment this ratio is high therefore it enlarges the range of insoluble drug amenable to encapsulation to include, allow targeting, of complexes to specific sites and reduce toxicity, for instance and membrane destabilizing agents. This extensive review explores the novel drug delivery for the cancer treatment.

Keywords: Liposomes, cyclodextrin, water-soluble drug, anti-cancer drugs

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1. INTRODUCTION

Some of the clinically effective drugs are hydrophobic in nature. However, to be pharmacologically active all drugs must possess some degree of water solubility, especially for parenteral administration. Therefore, to improve water solubility of drugs different strategies have been proposed. Cyclodextrins (CDs), produced from starch by means of enzymatic conversion, are one of the most versatile strategies in pharmaceutical technology, as an interest of formulation for a wide range of drug delivery systems from traditional dosage forms to novel drug carriers [1]. CDs are hydrophilic water-soluble oligosaccharides that contain hydrophobic cavities which can accommodate hydrophobic drugs. Their outer surface permits aqueous miscibility. The number of glucose units determines the name and size of the cone linked cavity: α CD, β CD and γ CD, having six, seven and eight glucose units respectively. In order to increase their aqueous solubility, some chemical modifications have been applied to obtain better water-soluble CD derivatives including hydroxypropyl β CD (HP β CD), sulfobutylether β CD (SBE β CD), methylated β CD (M β CD), and hydroxypropyl γ CD (HP γ CD). CDs can also increase the stability and even bioavailability of drug molecules by inclusion complex formation by a molecular complexation [2, 3]. No covalent bonds are formed during formation of the inclusion complex. Therefore, the binding force is relatively weak.

After administration, the rapid dissociation of drug/CD inclusion complexes takes place either because of dilution by the plasma and extracellular fluids or because blood components displace the included drug. Thus, the dissociated drug which is separated from its carrier will be rapidly cleared at the rate of free drug from blood circulation. The CD molecules are mainly excreted through the kidney [4], which may induce renal toxicity especially after chronic use [5, 6]. Another hard obstacle in the use of CDs is that some kinds of CDs, especially M β CD, can interact with the membrane components of living cells causing irreversible changes in physical stability. CDs can remove the components of human erythrocyte membranes (e.g., phospholipids, cholesterol and proteins) by forming inclusion complexes in aqueous solutions to destabilize the cell bilayer [7]. These structural damages may ultimately lead to the disintegration of cell membrane and the toxicity to the body [8].

Carrier systems can increase the residence time of drugs in the circulation, can be designed to target drugs to areas in need of action and can enhance the bioavailability of many sparingly water-soluble drugs [9, 10].

Liposomes as a biodegradable, low toxic and solubilized drug delivery system have been widely applied[11]. Hydrophilic drugs are encapsulated within an aqueous compartment of liposomes, whereas hydrophobic drugs are entrapped within the lipid bilayer. Liposomal formulations have shown an ability to enhance the pharmacokinetics and pharmacodynamics of encapsulated drugs[12] since these formulations can induce rapid uptake and long retention by the target tissues. Moreover, drugs incorporated in the lipid bilayers rather than in the aqueous core of the liposomes are prone to more rapid release. Thereby, a hypothesis has been proposed to avoid this shortcoming that a poorly water-soluble drug is first bonded with water soluble carriers and then the complexes are encapsulated in the aqueous compartment of liposomes. The entrapment of hydrophobic drugs in the aqueous core of liposomes as soluble inclusion complexes with CDs has been proposed as an interesting strategy in 1994, thus obtaining drug in CD in liposome (DCL) systems [13]. The encapsulation of the drug CD inclusion complexes into liposomes combines the advantages offered by both CDs and liposomes. CDs increase drug solubility and availability, preserve the liposomal structural integrity from drug molecules and insert into the lipid bilayer membrane, whereas liposomes prevent drug CD complexes dissociation due to dilution by the plasma or the facile renal excretion of CD molecules. Consequently, this strategy can be useful for a better control of the in vivo fate of hydrophobic drugs, avoiding the rapid release observed after conventional incorporation into the liposome lipid phase[14].

Poor aqueous solubility and rate of dissolution are the two critical factors that affect the formulation and development process of drugs and limit their therapeutic application(13). The drugs that are poorly soluble and belong to class II or IV of biopharmaceutical classification system, represents a major challenge [15]. Also, it is remarkable that most of the cytotoxic anticancer drugs belong to the BCS class IV which comprises substances with both low solubility in aqueous fluids and low apparent permeability [16]. Cyclodextrin (CD) complexation based nanocarriers for effective delivery of anticancer drugs came into existence [17, 18]. This paper simultaneously explores the utility of cyclodextrin complexation and nanotechnology as unique approach for development of drug delivery system.

2. LIPOSOMES FOR HYDROPHOBIC ANTI-CANCER DRUGS FOR CANCER TREATMENT:

2.1 Liposomes:

Liposomes are progressed to become a versatile carrier system with promising clinical applications several years later. Liposomes, are sphere shaped vesicles consisting of one or more phospholipid bilayer in which the hydrophobic acyl chains are opposite each other within the bilayer while the hydrophilic polar ends are placed along the outer rim.

The advantages of liposomes over the other carrier systems: it consists of wide range phospholipids with varying phase transition temperature (Tc) is available, providing choice between solid or fluid vesicles. Cholesterol can be incorporated into the bilayers affecting membrane fluidity and stability-important factors for parenteral use. Amphiphiles can also be included during preparation to produce liposomes with positive or negative surface charge, and appropriate ligands such as antibodies[19], certain glycoproteins neoglycoproteins and glycolipids can be attached in order to target liposomes to specific cell surface receptors[20]. All of these properties are applicable to liposomes of different sizes, small unilamellar (SUV), large unilamellar (LUV) and multilamellar (MLV), each with their own intrinsic characteristics and advantages. Anti-tumor have been encapsulated either in the aqueous or lipid phases of liposomes, and considerable progress in research and technology have ensured that liposomes have evolved from the theoretical to the practical.

Liposomal encapsulation technology (LET) is the newest delivery technique used by medical investigator[21, 22].

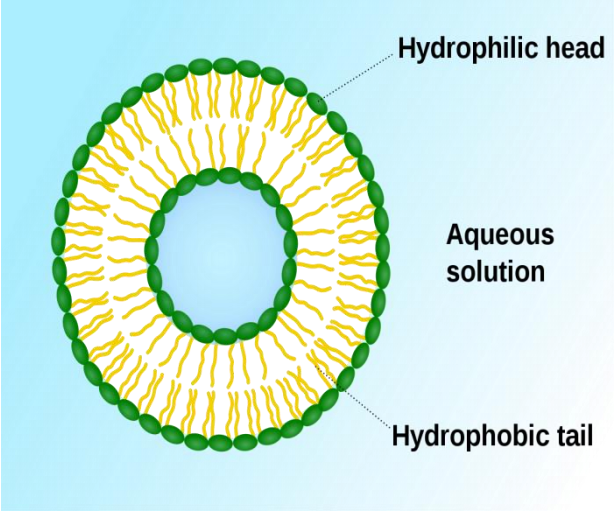

Figure 1: Structure of liposomes

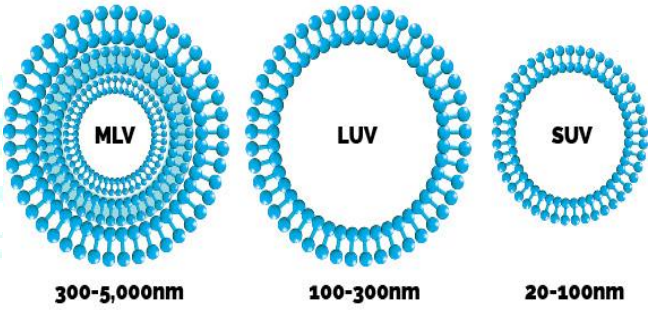

Figure 2: Types of Liposomal Systems

2.2 Properties of Liposomes:

Two types of liposomes. The bilayer membrane of liposomes is mainly composed of phosphatidyl choline (PC) is the amphipathic molecule and also known as lecithin in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains with a hydrophilic polar head group of phosphocholine. The length and saturation degree of PC acyl chains have an effect on the phase Tm of liposomal membrane. Above Tm, rotational isomerization occurs; thus, the lipid bilayers become more disordered. It was found that M b CD removes cholesterol preferentially from the disordered phase in giant unilamellar liposomes when it was above Tm [23]. In addition, the interactions between CDs and liposomes also depend on the lipid composition and the Liposome stability after incubation with different CDs was studied. Dried rehydrated vesicle (DRV), multilamellar vesicle (MLV) and small unilamellar vesicle (SUV) liposomes, composed of different lipids, were prepared for calcein encapsulation, and the encapsulation stability of calcein was tested by incubating with HP b CD, HP g CD or M b CD. It was found that M b CD had the most influence on liposome stability among these three CDs. It was also demonstrated that calcein release rates from vesicles were decreased in the order of MLV > DRV > SUV with the same lipid composition. The enhanced stability of SUV liposomes could imply that the smaller curvature of lipid membrane of SUVs (compared to DRV and MLV) did not allow the CD molecules to have

enough contact angle to interact with the lipid membrane[24]. Moreover, liposomes composed of saturated phospholipids (distearoylphosphatidylcholine [DSPC]) were found more stable compared to unsaturated egg phosphatidylcholine (EPC) liposomes, thus suggesting that phospholipid saturation and membrane rigidity have effect on the interaction between liposomal lipids and CD molecules.

3. CYCLODEXTRIN AS A SOLUBILITY ENHANCER:

Cyclodextrins are the cyclic oligosaccharides of glucopyranose units having a lipophilic central cavity and hydrophilic outer surface. Their molecules are large comprising a number of hydrogen acceptors and donors which do not permeate lipophilic membranes.The CDs are not perfectly cylindrical molecules but are toroidal or cone shaped due to lack of free rotation around the bonds connecting the glucopyranose units,

Depending on their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity. During drug CD complex formation no covalent bonds are formed or broken, and in aqueous solution, the complexes readily dissociate and free drug molecules remain in equilibrium with the molecules bound within the CD cavity. The parent or natural CDs consist of 6, 7 or 8 glucopyranose units and are referred to as alpha, beta and gamma CD, respectively. CDs containing 9, 10, 11, 12 and 13 glucopyranose units have also been reported. Hundreds of modified CDs have been prepared and shown to have research applications, but only a few of these derivatives, those containing the hydroxypropyl (HP), methyl (M) and sulfobutylether (SBE) substituents have been commercially used as new pharmaceutical excipients.

The aqueous solubility of many poorly soluble drugs is increased by forming inclusion complexes with their apolar molecules or functional groups. The resulting complex hides most of the hydrophobic functionality in the interior cavity of the CD while the hydrophilic hydroxyl groups on the external surface remain exposed to the environment. The net effect is that a water-soluble CD drug complex is thus formed.

4. DRUG IN CYCLODEXTRIN IN LIPOSOME:

4.1 Cyclodextrin based Liposomes:

Liposomes are concentric the vesicles containing an aqueous volume that is enclosed by membranous lipid bilayer. They entrap hydrophilic drug in the aqueous phase and hydrophobic drug in the lipid bilayer. In liposomes, cyclodextrin complexation competes with liposomal membrane binding, which tempers the potential benefit of complexation in prolonging hydrophobic drug retention [25, 26]. The entrapment of water-soluble cyclodextrin-drug inclusion complexes in liposomes leads to accommodation of insoluble drugs in the aqueous phase of vesicles, increases the drug to lipid ratio, enlarges the range of encapsulation, allows targeting of complexes to specific sites[27], and reduces toxicity. Some of the examples are discussed as under, in the year 2006, examined the antitumor effect of PEGylated liposomes of DOX complexed with γ-CD, administered through intravenous injection in BALB/mice bearing colon 26 tumor cells. Results reflected retardation in tumor growth, increase in drug retention, and improvement in survival rate [28]. Dhule et al. 2012 evaluated the liposomal curcumin's potential against cancer models of mesenchymal (OS) and epithelial origin (breast cancer). The 2-HP-γ-CD/curcumin liposome complex showed promising anticancer potential both invitro and in vivo. In another study, the antiproliferative and cytotoxic activity of anticancer agent LPSF/AC04in cyclodextrin complexed liposomes was enhanced[29]. Cui et al. 2011 developed stable PEGylated liposomal vincristine formulation with enhanced efficiency using sulfobutylether cyclodextrin as trapping agent. This formulation prolonged the circulation half-life from 43.6 to 70.0hrs and reduced toxicity [25]. Similarly, paclitaxel, a first line chemotherapy drug for ovarian cancer has also been entrapped in to cyclodextrin to I improve its solubility and the whole complex was loaded in liposomes [30]. Thus, this strategy can be incorporated into several other cyclodextrin complexed anticancer drugs into liposomes which can ultimately improve their retention inside vesicles.

4.2 Methods of preparations:

4.2.1 Two-step methods:

There are several methods for preparing DCLs which have different impact on the vesicle characteristics. Traditionally, the drug CD complexes are first obtained by kneading, spray drying, freeze drying, solvent evaporation or saturated aqueous solution methods. Then the complexes are further encapsulated into liposomal inner aqueous phase by different methods to obtain DCLs.

4.2.2 Thin-film hydration:

From different methods of preparing DCLs the most widely used method is the thin film hydration method [31,32]. The thin film is prepared by taking a dried solution of lipids at the bottom of a round flask, which is hydrated with drug CD complex solution. The extrusion of the above solution is done to reduce vesicle sizes. The drug CD complexes can also be added into the lipid bilayers of liposomes. The lipid film was obtained through evaporating a chloroform solution, which contains amphiphilic β CD and phospholipids, and then hydrated by buffer solution. It had been demonstrated that amphiphilic β CD can be mixed in any proportion with a typical mixture of phospholipids and cholesterol to provide stable, spherical and unilamellar mixed vesicles [33].

4.2.3. Dehydration rehydration:

The dehydration rehydration method, which has been demonstrated to allow an efficient entrapment of a wide range of drugs, is based on induction of preformed vesicles fusion by means of dehydration and controlled rehydration. For the preparation of DCLs, 1 palmitoyl 2 oleoyl glycerol 3 phosphocholine was dissolved in organic solvent and the solvent was evaporated to obtain thin lipid film. Then the film was rehydrated with water and the lipid suspensions were sonicated to reduce vesicle size. The resulting suspension was then mixed with 2 ml of 10 mM b CD aqueous solution and dried under vacuum at 40C. The rehydration was performed by adding 2 ml pH 7.4 PBS and incubating the mixture for 20 min at room temperature under vortexing[34]. 3,5 Bis (2-fluoro benzylidene) 4 piperidone (EF24) is a synthetic analog of curcumin that possesses potent anti-proliferative activity against a number of cancer cell lines. For EF24 containing DCL preparation, the dehydration rehydration method was used. Lipids (DSPC: cholesterol: dimyristoyl phosphatidylglycerol = 50:50:5, molar ratio) was dissolved in chloroform: methanol (2:1) and a thin film of lipids was obtained after evaporation of the solvent. Then the film was rehydrated with water, maintaining the total lipid concentration to 12 mM. The resulting suspension of MLVs was subjected to eight freeze thaw cycles. A cycle consisted of snap freezing the suspension in liquid nitrogen followed by immediate thawing in a 58C water bath. A sterile aqueous solution of EF24 HP b CD complex was added to the liposomal suspension and the mixture was vortexed for 30 s and diluted with pH 7.4 PBS to a lipid concentration of 2 mM. It

was found that about 19% of the EF24 HP b CD complex was encapsulated inside the liposomes (320.5 ±2.6nm) by this technique. With extrusion technique, the size of 177 ± 6.5 nm was obtained without any effect on EE [35].

4.2.4 Active loading:

The commercial impact of liposomes is strengthened by the invention of several active drug encapsulation methods, allowing the encapsulation of several weak base or weak acid drugs with very high drug to lipid ratios [36]. For the preparation of doxorubicin loaded DCL, pH gradient method was used. Blank liposomes (lipid composition: DSPC/cholesterol/ 1,2distearoyl glycerol 3phospho ethanolamine N (carbonyl methoxy polyethylene glycol) [DSPE PEG], inner aqueous composition: 300 mM citric acid pH 4.0) were prepared by freezing thaw method. The pH of the liposome suspension was raised from 4.0 to 7.8 with 1 M NaOH. Then doxorubicin γ CD solution was added at the drug : lipid weight ratio of 0.2. Doxorubicin loaded CL was also prepared by the same technique. The EE of doxorubicin DCL and CL were determined to be 91.1 ± 1.1 and 96.4 ± 0.5%, respectively[37]. Gefitinib, an antitumor agent, was encapsulated into liposomes by ammonium sulphate gradient method. The liposomes were composed of HSPC: cholesterol: DSPE PEG (55:45:5, molar ratio). After adding 0.1M HP b CD in 300 mM (NH4)2SO4 solution, the EE values significantly increased from 71.6 ± 1.7% to 85.5 ± 1.2%. CDs can form inclusion complexes with drug molecules in inner aqueous media of liposomes, hence the increase of drug solubility and trans membrane concentration difference [38,39]. In addition, novel SBE β CD gradient method was proposed and SBE β CD anions inside liposomes were found to provide the driving force for the loading of drug, because they could complex with protonated drugs to improve drug retention [40]. Active loading is the preferred method for optimizing the loading of ionizable drugs in liposomes as measured by drug to lipid ratios, but the extremely low aqueous solubilities of many hydrophobic drug candidates may limit the external driving force, thus slowing liposomal uptake during active loading. Therefore, the addition of CDs can improve the solubility and the drug loading efficiency of liposomes[41].

4.2.5 One-step method:

For the preparation of DCLs, the two step method was tedious due to two reasons. First, the preparation of drug CD soluble inclusion complexes need a long time (e.g., 48 h). Second, the entrapment of inclusion complexes into liposomes was usually performed by thin film hydration method, limiting industrial production abundantly. The spray drying process is considered as a single step, fast procedure applied in the formulation and processing of bio pharmaceuticals. For the preparation of DCL, the solution for spray drying was prepared by suspending lecithin (4.601 g), mannitol (0.460 g; presieved), drug (metronidazole [1.026 g] or verapamil chloride [7.66 g]) and HP b CD (9 g) in chloroform (200 ml). The mixture was sonicated for 8 min (bath sonicator) and subjected to spray drying. The dried product was hydrated with pH 7.4 PBS stirring for 45 min to obtain DCL. It was found that liposomes prepared by the one step spray drying method were of regular spherical shape regardless of the presence of the drug or CD. No morphological difference was observed between empty liposomes and liposomes which contain drug, CD or both[42]. A simple and fast one step method was also performed to prepare honokiol in HP b CD in liposome[43]. First, 2.0 mmol HP b CD was dissolved in dehydrated alcohol to obtain 2.5% (m/v) solution and kept for 0.5 h to let it freely swell. Second, 1.0 mmol honokiol was added into the solution and the inclusion complex solution was formed by self-association after stirring for 1 h at room temperature. Third, SPC (1.500 mmol), cholesterol (0.4750 mmol), DSPE PEG (0.0075 mmol) and vitamin E (0.0175 mmol) were added into the inclusion complex solution and the mixture was stirred at 60C for the dissolution of lipids. Meanwhile, 2.5% (m/v) aqueous phase sucrose solution was heated in a water bath at 60C. Fourth, organic phase was slowly injected into aqueous phase using a 5 ml syringe under the stirring rate of 600 rpm and DCL was formed (Figure 2). Then, the sol vent was evaporated under vacuum in a rotary evaporator. After diluted with 2.5% (m/v) sucrose solution, the suspension was freeze dried. The final molar ratio of honokiol: HP b CD: lipid was 1:2:2. In the preparation process, the preparation of honokiol in HP b CD could be finished in 1 h through simple dehydrated alcohol soluble inclusion complex method. Meanwhile, the preparation of liposomes was performed through simple and fast ethanol injection method. Therefore, this whole method of preparing DCL was easy to scale up. Although the one step method has such advantages as simple, economic and time saving, the form and location of drugs in liposomes prepared in this way should be clearly demonstrated.

4.3 Characterization:

4.3.1 Vesicle size:

The effect on vesicle size depends on liposome type. The encapsulation of drug CD complex resulted in an appreciable size increase for MLV, frozen and thawed MLV and large unilamellar vesicles (LUV). Celecoxib, a nonsteroidal anti-inflammatory drug, was encapsulated into MLVs in the form of free drug or drug β CD complex. The corresponding vesicle sizes were determined to be about 1.32 ± 1.04 µm and in the range of 6.52 ± 1.54 10.42 ± 0.44 µm, respectively[44]. But for SUV, no significant size modification was observed in the presence of CD, not even when the complex concentration was increased. It was reported that the entrapment of drug molecules in the lipid bilayer or the encapsulation drug CD inclusion complexes in the inner aqueous phase did not modify the size of the SUV [45]. For blapachone, an antitumor agent, the drug loaded CL in the suspension dosage forms presented vesicle sizes of 104 ± 2.33 nm, whereas the DCL under the same circumstance presented vesicle sizes of 112 ± 1.49 nm, respectively [32]. The pore size of the membrane determined the particle size of the liposomes, on which the presence of CD can have little effect. Sometimes the enhancement of the vesicle size during storage is found, which means that the stability of DCL is probably unsatisfactory. A hypothesis of the DCL particle size enhancement is that CDs could replace the drug molecules for the lipid components, especially cholesterol, from liposomes by forming inclusion complex with them. Thus, it could destabilize the bilayers of DCLs.

4.3.2 Entrapment efficiency:

The amount of hydrophobic drug incorporated into the CL bilayer is often limited in terms of drug to lipid ratio. Entrapping inclusion complexes in liposomes has been proposed to increase the EE and stability of liposomes compared with CLs. For EF24, an analog of curcumin, the conventional thin film hydration method provided only 0.4% EE which increased to 1% when a reverse phase evaporation method was used for CL preparation. It was obvious that lipid compartment of the liposomes was not able to host EF24, and the aqueous solubility of EF24 was not large enough for its accommodation inside the aqueous core. EF24 solubility in water increased remarkably from 1.64 to 13.76 mg/ml after complexation with HP β CD. And the complexes were further encapsulated into liposomes. The EE of EF24 DCL was determined to be 19.7%. The enhancement in EE could be attributed to the encapsulation of more soluble

EF24 complexes into the aqueous liposomal core which provided a relatively larger volume than lipid bilayers. The type of CDs could affect the EE values of DCLs. For ketoprofen containing DCLs, the complex with HP β CD,in virtue of its greater stability than the complex with β CD, allowed higher EE and gave rise to more stable MLV systems [46]. As for SUV, HP β CD also seemed to be superior to β CD in consideration of drug loading capacity. The drug: lipid ratios were determined as 0.321, 0.102 and 0.778 for prednisolone, prednisolone: β CD and prednisolone: HP β CD containing liposomes composed of EPC and cholesterol (1:1, molar ratio) [47]. For DCL, the EE also depended on both the liposome preparation method and the complex concentration in the aqueous phase and was in the order of MLV > LUV > SUV. SUV vesicles showed the lowest EE value compared with the other types of DCLs. The reduced volume of the aqueous compartment, with respect to the other formulations, due to the smaller dimensions of these unilamellar vesicles can be considered as the main factor responsible for this result[48]. In addition, EE value increased with the increase of the HP β CD complex concentration up to 10 mM. Moreover, it was not possible to use higher complex concentrations, due to the destabilizing effect of CDs toward the liposomal membrane[48].

4.3.3 Release profile Encapsulation of drug:

CD complexes into inner aqueous phase of liposomes could result in a modulation of the in vitro kinetics of release of drug molecules. In most cases,

DCLs presented a more prolonged release profiles compared to CLs. The slow release pattern of the DCLs may be attributed to the fact that there are more barriers to the diffusion of drug from the DCLs (44). The release profiles of three different usnic acid loaded formulations (inclusion complex, CL, DCL) were compared. Usnic acid in inclusion complex released practically 50% of usnic acid at 3 h, corresponding to the initial burst effect, followed by a linear release of 80% at 11 h, reaching 99.1% in 30 h. The release of usnic acid from CL reached 30% within 7 h and 55% in 24 h, achieving 70% in 72 h, whereas the release of usnic acid from DCL was significantly slower (32.5% at 24 h) than from CL (50% at 20.5 h and 65% at 33 h) . For liposomes containing amphotericin B, amphotericin B HP β CD complex and amphotericin B SBE β CD complex, 65.1 ± 8.5, 12.2 ± 3.6 and 11.8 ± 3.4 % drug molecules were found released from vesicles at 60 min in pH7.4 PBS, respectively [49]. As for DCL, two routes may account for the drug release. One is that drug CD inclusion complexes are transported from inner aqueous phase to lipid bilayers, and then the whole inclusion complexes are released[50]. The other way is the release of free drug, which is in equilibrium with the inclusion complexes in inner aqueous phase of DCLs. The release rate is affected by a constant of inclusion complexes. Whether the release process follows one route or the other, the lipid bilayer barrier should be overcome at first[49]. Since HP β CD entrapped in the aqueous compartment of liposomes was found to be membrane impermeable[51], the latter way might be the main drug release route for DCL containing drug HP β CD complexes.

4.3.4 Pharmacokinetics:

If drug CD complexes are injected directly, rapid dissociation of the complexes takes place because of either dilution or dis placement of the included drug by other blood components. The released drug is then distributed and metabolized as free drug, while the CD moiety is excreted through the kidneys. Therefore, the improvement of pharmacokinetics in vivo following complexation with CDs might be limited. In addition, after intravenous administration of naked drug CD complexes, interaction of CD molecules with cholesterol of red blood cells may lead to drug displacement from the complexes and subsequent haematological toxicity. For curcuminoid 4 [3,5 bis (2chlorobenzylidene 4 oxo piperidine 1yl) 4 oxo 2butenoic acid], a novel antitumor agent, if intravenously injected in the form of HP β CD inclusion complex, renal clearance of complex accounting for > 50% elimination within the first 10 min after administration was observed. Therefore, DCL was selected for the drug because liposomes were eliminated through a relatively slower reticuloendothelial route which can be further delayed by altering the liposomal composition [52]. DSPC liposomes containing entrapped complexes of 14C labelled HP β CD with 3H labelled drugs were prepared. Intravenous injection of free complexes into rats led to their rapid clearance from the circulation with up to 94% of HP β CD recovered in 24 h urine together with lesser and variable amounts of drug (up to 46% of the dose). After injection of DCLs, only 6 13% of HP β CD and a moderate proportion of drugs (up to 26% of the dose) were recovered in 24 h urine. Most of the HP β CD was recovered in the liver (up to 83%) and spleen (up to 13% of the dose) 30 min after injection, together with a variable proportion of drugs. Therefore, compared to drug CD complexes, administration of DCLs could serve as a better means to control the action of a wide range of therapeutic agents. Encapsulation into DCLs can lead to significantly improved pharmacokinetic properties of hydrophobic drugs. The results of plasma pharmacokinetics revealed that free honokiol was quickly removed from the circulating system after intravenous injection. However, honokiol in HP β CD in liposome significantly retarded the elimination and prolonged the residence time in circulating system. Indomethacin DCLs were obtained by encapsulating indomethacin b CD or HP β CD inclusion complexes into liposomes. After intravenous injection, for indomethacin CL (encapsulated into lipid bilayers), T1/2b and AUC0 -- ¥ values were determined to be 75.4 ± 13.6 min and 3904 ± 23.4 μg/ml min, respectively. For b CD DCL, the corresponding values were determined to be 106.8 ± 16.8 min and 5808 ± 50.9 μg/ml min, respectively. And for HP b CD DCL, the corresponding values were determined to be 122.7 ± 63.1 min and 6290 ± 150.7 μg/ml min, respectively[53]. Considering that encapsulation of drug CD complexes into liposomes results in a slow release profile in vitro, therefore, the systematic circulation time of the drug is prolonged[54].

4.3.5 Antitumor activity:

LPSF/AC04, (5Z) [5acridin 9ylmethylene 3 (4 methyl benzyl) thiazolidine 2,4dione], is an acridine based derivative. Evaluated with human breast cancer T47D cells in vitro, the IC50 values of 82.57 ± 9.26 and 65.06 ± 5.95 μM were found for free LPSF/AC04 and LPSF/AC04 CL (encapsulated into lipid bilayers), respectively. On the other hand, IC50 values of 35.00 ± 13.6 and 32.00 ± 14.8 μM were found for the liposomal formulations containing inclusion complexes (LPSF/AC04 HP β CD and LPSF/AC04 HP g CD), respectively. These results clearly demonstrated that the incorporation of LPSF/AC04 CD inclusion complexes into liposomes increased the cytotoxicity activity by twofold over LPSF/AC04 loaded liposomes [45]. It was concluded that DCL strategy was an alternative means of overcoming drug's poor solubility, as well as probably improving drug penetration into the cells and enhancing drug antiproliferative activity. Antitumor efficacy against human lung adenocarcinoma NCI H441 and prostate cancer PC 3 cell lines in vitro was investigated and compared between EF24 loaded DCL and free drug. In general, it was observed that EF24 DCL exceeded the antiproliferative activity to plain EF24 ($p < 0.05$) . Following intravenous injection to the tumor bearing mice, the AUC

value of DOX in solid tumors up to 72 h in the PEGylated DCL system was ~ 2.3 times higher than that of the PEGylated CL system. The AUC values of DOX in plasma were determined to be 1338 ± 97 and 2090 ± 54 µg h/ml for DOX CL and DCL, respectively[37]. Enhanced AUC levels of drug in plasma and solid tumors after intravenous injection of DCL or CL could be attributed to the slow release profiles of the PEGylated DCLs. Consequently, the improved antitumor activity in vivo of DCLs was expected compared to free drug or drug loaded CLs. Nevertheless, the antitumor activity of DCLs in vivo should be intensively investigated. In addition, to increase efficacy of chemotherapeutic drugs, toxicity of the drugs could also be reduced through DCL. Following intravenous administration of trans dehydrocrotonin (T DCTN) suspension, the microsteatosis and cell degeneration were more pronounced in the hepatocytes of mice[55]. There were few changes in animal livers treated with T DCTN loaded CL and even fewer of those treated with DCL. The decrease in the hepatotoxicity of mice treated with CL and DCL might be due to a lower concentration of drug in the liver.

4.3.6 Transdermal absorption:

The clinical application of liposomes can carry a risk of acute immune toxicity manifested in hypersensitivity reactions that do not involve IgE but arises as a consequence of activation of the complement system [56]. Therefore, although no immune toxicity is reported until now, more attention should be paid to the similar risk of the application of DCLs. CLs have been proved having little value as carriers for transdermal drug delivery, because they are generally accumulated in the upper layers of the stratum corneum (SC) with minimal permeation to deeper tissues or the systemic circulation [57]. With the incorporation of edge activator in the lipid bilayer structure, a new type of liposomes, elastic liposomes, was first introduced by Cevc and Blume in 1992 [58]. Due to the deformability, elastic liposomes are claimed to be able to squeeze through channels one tenth the diameter of the vesicles [59], allowing them to penetrate across SC by the inter cellular route. Elastic liposomes have been widely applied for the transdermal drug delivery [60]. Edge activator is usually a kind of surfactant, which destabilizes lipid bilayer of the elastic liposomes and increases elasticity of the bilayer simultaneously. Among edge activators, sodium cholate, sodium deoxycholate, Span 80, Tween 80 and Tween 20 were commonly used. In 2008, elastic DCLs were first reported by Jain et al. for the transdermal delivery of meloxicam, an anti-inflammatory agent. The results of in vitro skin permeation studies revealed that elastic DCL enhanced skin permeability compared to elastic CL and free drug. The maximum flux obtained from meloxicam loaded elastic DCL was substantially 9.1 times higher than that of plain drug and nearly 1.4 times higher than elastic CL. Theoretically; the entrapment of drug CD complexes into elastic liposomes will increase the advantage of both of them. On one hand, CD plays a role as a solubilizer agent, a chemical protector and sometimes a permeation enhancer [61]. On the other hand, the elastic liposome seems to be able to penetrate into the intact skin when it is administered under nonocclusive conditions. Results of skin permeation and deposition study showed that encapsulation of isotretinoin HP β CD complex into elastic liposomes significantly increased the skin deposition. At the end of skin permeation experiments in vitro (24 h), 1.2, 3.0, 16.1 and 26.2% drug deposition were found in skin for isotretinoin solution, HP β CD complex, elastic CL and elastic DCL, respectively. Besides good reservoir effect, the flux across skin in vitro was also found to be enhanced by elastic DCL[62]. The deformability of liposomal vesicles was not affected by the encapsulation of drug CD complexes [63]. Moreover, the vesicle characteristics could be improved by DCL strategy. The percentage of β methasone released was 20.9 ± 1.6% for classical liposomes encapsulating β methasone HP γ CD inclusion complexes versus 44.7 ± 3.1% for elastic ones. The percentage of b methasone released was 76.5 ± 2.1% for classical liposomes containing b methasone in the lipid bilayer and 82.7 ± 4.1% for elastic ones [30]. Based on these results, it is proved that the strategy of DCL improves the retention of β methasone inside the liposome cavity, which might account for the good reservoir effect of elastic DCLs. However, for some drugs, encapsulation into elastic DCL has little effect on the vesicle properties compared to elastic CL. There is no statistically significant difference between the physicochemical parameters of elastic liposomes with quercetin and resveratrol entrapped or encapsulated as CD inclusion complexes[64]. To obtain stable elastic DCL, CDs should have a higher affinity for drug molecules than for membrane lipids activators.Thus the entrapment of highly concentrated drug CD inclusion complex into liposomes will has no or only minimal interaction with lipid components of the liposomal membrane. Therefore, if CD has lower affinity for the drug than the lipids or edge activators, the vesicle proper ties could not be improved by elastic DCL strategy. Subsequently, the improved skin permeation behaviour could not be expected.

CONCLUSIONS

It has been observed through this review the liposomes and the cyclodextrins as drug delivery systems have contrasting characteristics, interaction with biological environment, mode of dug delivery and entrapment. However, these two systems are improved over the years. Liposomes are designed with predetermining optimal drugRelease and diverse circulatory residence times, similarly hydrophilicity increased by derivatisation in Cyclodextrins and it became less toxic. Therefore, Cyclodextrins bioavailability and therapeutic efficiencies are improved. There are certain disadvantages of each system but it can be cured by the entrapping cyclodextrin complexes into liposomes to produce a single system and preliminary resultsare promising.Work needs to be done to optimize the system in order to understand the factorsinvolved in drug displacement and to establish conditionsof entrapment whereby this can be reduced.The stability and entrapment behavior of additionaldrug/cyclodextrin complexes also needsinvestigation.

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