Nanoliposome Potentials in Nanotherapy: A Concise Overview

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Abstract:
Liposomes have attracted great interest as efficient carriers for nutrients, drugs and other bioactive agents as well as ideal models for biological membranes. This article intends to provide an overview of liposomes and nanoliposomes definition as well as their properties and preparation methods. Also it elaborates on various applications of nanoliposomes in nanotherapy including diagnostics, targeted cancer and gene therapy. Finally structure and function of liposomes and nanoliposomes are compared with other lipidic nanocarriers.
Keywords: *Liposome, Nanoparticle, Nanotherapy; Nanoliposome; Lipidic carrier, Bioactive delivery*

1. INTRODUCTION

Liposome science and technology are one of the fastest growing scientific fields due to contributing to delivery of cosmetics, food and drug, as well as study the structure and function of biological membranes and origin of life. The word liposome has been adopted generally to describe mesomorphic lipid-water structures, which chemical components are lipids and/or phospholipids. When phospholipids such as lecithin are present in water, by sufficient uptake of energy form a series of closed bilayers vesicle named liposome (to shield the hydrophobic groups from the water while still maintaining contact with the aqueous phase via the hydrophilic head group). Lipophilic molecules (e.g. cyclosporin A, vitamin E, Phenytoin) can also be incorporated into liposomal membranes or may be complexed with cyclodextrins and subsequently encapsulated within the liposome aqueous compartment [1]. The surface charge of liposomes can be neutral, negative or positive in physiological pH ranges.

According to the number of lamellae, size, and preparation method, phospholipid vesicles can be classified into the following groups [2-4]: SUV: small unilamellar vesicles (20-50 nm); LUV: large unilamellar vesicles (>560 nm); MLV: multilamellar vesicles (170-5000 nm); OLV: oligolamellar vesicles; MUV: medium-unilamellar vesicles (unilamellar vesicles; >100 nm); GUV: giant unilamellar vesicles (cell size vesicles with diameters >1 μm); REV: single or oligolamellar vesicles made by reverse-phase evaporation; MLV-REV: multilamellar vesicles made by the reverse-phase method; SPLV: stable plurilamellar vesicles; FATMLV: frozen and thawed MLV; VET: vesicles prepared by extrusion technique.

Liposomes are especially suitable for gene transfer in vitro and in vivo due to their low level of toxicity

and immunogenicity [5-7]. Not only liposomes serve as unique model membranes and nucleic acid delivery vehicles, they have been also reported to be used as delivery systems of enzymes [8], drugs, hormones, blood factors, antigens, diagnostic materials, vaccines and cosmetics [9].

For mentioned applications liposomes should have high encapsulation efficiency, long-term stability ideal release properties and narrow size distribution. Only a small number of liposomal products have been approved for human use so far, due to toxicity of some formulations, low entrapment efficiency, instability and high cost of production especially in scale up [9].

Several bench scale and a few large scale techniques have been presented for liposome preparation giving rise to vesicles of different diameter sizes (from 20 nm to several microns) and number of bilayers [10, 11, 1, 3, 4]. However, most of these techniques are not suitable for the encapsulation of sensitive substances because of their exposure to the mechanical stresses (e.g. sonication, high pressure or shear tension), potentially harmful chemicals (e.g. organic solvents and detergents) or change of pH during the preparation. Conventional liposome preparation techniques were discussed extensively by Gregoriadis [1, 11] and New [4]. More novel preparation techniques are introduced with their own advantages and disadvantages. In liposomes preparation methods emphasis is not towards assembling the membranes, but towards getting the membranes to form vesicles with right size, structure, and efficiency without any leakage. Mozafari described a new method for fast production of liposomes without the use of any hazardous chemical or process has been described. This method involves the hydration of the liposome components in an aqueous medium followed by the heating of these components, in the presence of glycerol (3% v/v) [12-15]. There are indications that this heating method simulates the formation of the early cell membranes under the conditions of the primordial earth [16].

This review focuses on the potential of nanotechnology in liposome and nanoliposome applications, including the recent status of nanocarriers for bioactive delivery and diagnostics as well as targeting strategies and gene therapy. Also the characterization of anionic liposomes prepared by the heating method in terms of their entrapment efficiency will be presented.

2. NANOLIPOSOME

The term nanoliposome has recently been introduced to exclusively refer to nanoscale lipid vesicles [9] while liposome refer to a lipid vesicles with diameter range from around 20 nm to several micrometers. Nanoliposomes possess the same physical, structural, thermodynamic properties manufacturing and mechanism of formation as the liposomes (described above). The underlying mechanism for the formation of liposomes and nanoliposomes is basically the hydrophilic–hydrophobic interaction between phospholipids and water molecules [17-19]. Vesicles prepared in nanometric size ranges may end up becoming micrometric particles upon storage due to aggregation. However, nanoliposomes should have sufficient stability to maintain their sizes and could be defined as 'bilayer lipid vesicles possessing and maintaining nanometric size ranges during storage and application' [9]. Targeted therapy can also be achieved efficiently via liposomes and nanoliposomes employing passive or active targeting mechanisms [9, 17-19].

Active targeting is achieved by engineering carriers sensitive to different stimuli (e.g., pH, temperature, light, etc.) or conjugating the bioactive/carrier system to one or more targeting ligands such as tissue or cell-specific molecules. Zhang et al. [20] linked a PEGylated (treated with polyethylene glycol) liposomes to a monoclonal antibody for the human insulin receptor and showed widespread reporter expression in the brains of rhesus monkeys. Passive targeting uses the natural course followed by the bioactive–carrier complex after introduction to the body as the method of site-specific delivery and release of the bioactive agent. So it is based on physio-anatomical conditions of the body and the physicochemical properties of bioactive–carrier complex. The clearance kinetics and in vivo biodistribution of carrier systems depend on the physicochemical factors like size, charge and

hydrophobicity and can be manipulated to enable passive targeting.

3. APPLICATIONS OF NANOLIPOSOMES IN NANOTHERAPY

Bioactive nanocarriers (in general) and drug delivery (in particular) constitute a significant domain of nanomedicine. New bioactive materials require novel delivery technologies to minimize side effects [21]. The delivery of some bioactives to special sites in the body and their release behavior is directly affected by particle size. Nanocarriers have the potential to increase solubility, enhance bioavailability, improve time-controlled release and enable precision targeting of the entrapped compounds to a greater extent due to more surface area [22]. As a consequence of improved stability and targeting, the amount of materials required for a specific effect when encapsulated in, or incorporated to, a nanocarrier is much less than the amount required when unencapsulated. This is particularly useful when dealing with expensive/rare bioactive materials. A timely and targeted release improves the effectiveness of bioactive compounds, broadens their application range and ensures optimal dosage, thereby improving cost effectiveness of the product. In general, reactive or sensitive material, such as polynucleotides and polypeptides, can be turned into stable ingredients through encapsulation or entrapment by nanocarrier systems [22].

Novel nanocarriers can encapsulate certain toxic materials (e.g. chemotherapy agents) [21] or new biomacromolecules (e.g. blood proteins and biovaccines). The success of gene therapy (with DNA and RNA transfer) depends on the innovative bioactive delivery methods [23]. The importance of delivery technology is exemplified by the presence of more than 300 USA companies which are involved with developing bioactive delivery platforms [24]. Micro-carriers can be effective for bioactive targeting to certain parts of the pulmonary tract while nano-carriers are more useful in targeted cancer therapy [25]. Current methods of preparing nano-/micro-particles are mainly based on the double emulsion methods or solvent exchange technique [26]. The main problems with these methods are the low drug loading capacity, low loading efficiency, and poor ability to control the size distribution. The use of nanoliposomes could allow high loading efficiency and monodisperse size distribution [25].

4. NANOTECHNOLOGY IN BIOACTIVE DELIVERY

Nanoscience has a profound impact on disease prevention, diagnosis, and treatment [27-30]. Its applications in medicine include molecular imaging, diagnosis, bioactive encapsulation and passive or active targeted delivery. Some of the current nanocarrier systems are nanoscale size of conventional systems, such as nanocrystals, micelles, nanoliposomes and dendrimers [25]. Degradable compounds, such as peptides and polynucleotides are used in new delivery systems to protect and improve the pharmacokinetics of delivery. Also increase bioavailability, improve the controlled release and enable precision targeting [31, 32]. Nanocarriers can be applied for pulmonary therapies [33], as gene delivery vectors [34-36], and for stabilization of sensitive biomaterials [37, 38]. They reduce toxicity and increase efficient distribution of bioactive materials [39]. Nanocarriers help to penetrate or overcome blood brain barrier, branching pathways of the pulmonary tract, and the tight epithelial junctions of the skin [21]. Micelles are under investigation as carrier vehicles of poorly soluble, hydrophobic bioactives [40]. Microemulsions have also been investigated for their potential to serve as a drug carrier vehicle, since their oil phase can contain a high payload of hydrophobic drugs [41, 42]. The use of lipidic carriers with high lipid-phase to water-phase ratio, such as onion-shaped liposomes in the form of multilamellar vesicles is another possibility for the encapsulation and controlled release of lipid soluble agents.

4.1. Site-specific bioactive delivery

Bioactive targeting of various nanoproducts is possible via anatomical changes and

pathophysiological conditions of abnormal damaged tissues [43, 44]. An ideal targeting system has long circulation time, is present at sufficient concentrations at the target site, and loses its therapeutic activity in circulation [45]. The increased vascular permeability as well as impaired lymphatic drainage in tumors offers opportunity of extravasations of the nanosystems and their selective localization in the inflamed tissues [46-51]. The tendency of nanosystems to localize in the reticuloendothelial system also presents an excellent opportunity for passive targeting of therapeutic agents to the macrophages present in the liver and spleen in candiasis, leishmaniasis, etc. [52, 53]. The therapeutic value of many promising bioactives for the treatment of various neurological disorders is diminished by the presence of the blood-brain barrier [54, 55]. Nanoparticles can be effectively used to deliver relevant therapeutics to the brain [56, 57] and leads to a more selective delivery to improve therapeutic efficacy and reduces toxicity [58-61]. Visser et al. [62] studied targeting of pegylated liposomes loaded with horseradish peroxidase and tagged with transferrin to the blood-brain barrier in vitro. The results showed the effective targeting of liposomes loaded with protein or peptide drugs to the brain capillary endothelial cells. Enhanced uptake efficiency has also been shown for gastrointestinal absorption [63, 64] and transcutaneous permeation [65], with particles around 100 nm and 50 nm in size, respectively. Also biodegradable nanoparticles of gelatin and human serum albumin show promise for bioactive delivery to the lungs [66]. Skin acts as a key target as well as a principle barrier for topical/transdermal (TT) bioactive delivery. This drug administration avoids the hepatic first pass effect, provides continuous drug delivery, decreases side effects and improves patient compliance [67]. A major obstacle in TT delivery is low percutaneous penetration [68]. Several approaches have been used to weaken skin barrier and improve TT delivery [69, 70, 30]. The nanotechnological approaches offer application of elastic vesicles and ethosomes in nanoscale size [70-72] detect the effect of nanoliposomes (ca. 200 nm average size) in the protection of stratum corneum (SC) against a nonionic surfactant. The imaging technique enabled visualization of native and treated SC (incubated with nanoliposomes and octyl glucoside) without causing damage to the SC during sample preparation for the microscopic investigations [72].

4.2. Nanotechnology in polynucleotide delivery

A new class of bioactive therapeutic agents are based on the polynucleotides which have the potential to offer healing of human (and animal) diseases at their cause rather than only treating their symptoms [18]. Polynucleotide-based therapeutics includes antisense, antigene oligonucleotides, ribozymes, DNAzymes, DNA and RNA aptamers and small interfering RNA (siRNA) [73, 36, 74, 75]. Although nucleic acid drugs are in the early stages of clinical trials, they can be considered as promising therapeutic agents for therapy of diseases such as hereditary disorders, cancer, neurological and cardiovascular disorders, AIDS and other viral infections [18, 74, 75].

Since 1980, more than 400 clinical studies in gene therapy have been reported [76]. Delivery vectors are used in gene transfer because of limited ability of naked DNA transfer to cells due to susceptibility to enzymatic degradation. Cationic liposomes and nanoliposomes are the most used nonviral vectors frequently applied in human gene therapy [77-79]. The ability of them to mediate transfection was attributed to spontaneous electrostatic interaction between them and negatively charged DNA molecules that ensures an efficient condensation of the polynucleotides. Modification of the lipid composition causes an appropriate charge of liposome–polynucleotide complex to increase possibility of cellular uptake. Proposed mechanism of oligonucleotide uptakes from cationic liposomes are fusion and endocytosis [80]. An effective in vivo gene transfer can be achieved by improving the biological and the physicochemical properties of the liposomes/DNA complex. Application of anionic nanoliposomes as polynucleotide delivery vehicles has been extended because of the toxicity and some other complications associated with the cationic agents [18, 35, 36]. A method of incorporating polynucleotides to anionic liposomes mediated by

divalent cations has been reported by Mozafari et al since 1994 [81-85, 35]. This group studied the structure of the ternary complexes of liposome–Ca^{2+}–DNA morphologically using scanning probe and other microscopes [83, 85]. In addition, the mechanism of calcium-induced DNA interaction with liposomes containing zwitterionic lipids, as well as those containing anionic lipids, has been studied using light scattering [84] and different microscopic techniques [83, 85]. The problems of toxicity and scale-up have been addressed by a new technique, called the heating method, [18, 35], in which no potentially toxic solvent or deleterious procedure is involved. Application of nonviral nanoparticles (usually 50–500 nm in size) for transfection of plasmid DNA has been reviewed [53] with emphasis on replacement of viral vectors by potentially less immunogenic nanosize polynucleotide carriers.

5. OTHER NANOLIPIDIC CARRIERS

A lipidic carrier can encapsulate a compound and inhibit its degradation from free radicals, metal ions, pH and enzymes [86]. They can accommodate water and lipid soluble agents, providing a synergistic effect [87]. Another property of lipid-based nanocarriers is the targeted delivery both in vivo and in vitro conditions via active (e.g., by incorporation of antibodies) and passive (e.g., targeting based on the particle size) mechanisms [9]. The main lipid-based nanoencapsulation systems that can be used for the protection and delivery of various bioactive materials are explained below.

5.1. Archaeosomes and nano archaeosomes

Archaeosomes are liposomes made from one or more of the polar ether lipids extracted from the domain Archaea (Archaeobacteria) whose native environments include high salt concentrations, low pH values or high temperatures. Compared with liposomes (which are made from ester phospholipids), archaeosomes are relatively more thermostable, and more resistant to oxidation and chemical and enzymatical hydrolysis. They are also more resistant to low pH and bile salts that would be encountered in the gastrointestinal tract [88]. The core lipids (polar head groups removed) of archaea consist of archaeols (usually 20 carbons per chain) and caldarchaeols (40 carbons per chain), wherein the regularly branched, 5-carbon repeating units forming the isoprenoid chains are attached via ether bonds at the sn-2,3 position of the glycerol carbons. In contrast to this, the core lipids found in bacteria and eucarya consist of unbranched unsaturated fatty acyl chains, attached via ester bonds to the sn-1,2 glycerol carbons. The polar moieties are similar to those (phospho, glyco, polyol, amino, hydroxyl groups) encountered in ester lipids, but phosphatidylcholine is rarely present in archaeal lipids [89, 88]. Although archaeosomes are a recent technology, they have already proven to be a safe delivery system for bioactive agents including drugs and vaccines [90]. Archaeosomes prepared from the total polar lipid extract or from individual purified polar lipids show promise as adjuvants that promote strong humoral and cytotoxic T-cell responses to encapsulated soluble antigens. As is the case with liposomes, it is possible to incorporate ligands such as polymers to archaeosomes. Incorporation of polyethyleneglycol and coenzyme Q10 into archaeosomes improve the tissue distribution profiles of intravenously administered vesicles [91]. It has recently been reported that intravenous and oral delivery of nano archaeosomes to an animal model was well tolerated with no apparent toxicity [92]. The results of these studies are very promising for the utilization of archaeosomes in the encapsulation and delivery of different bioactive compounds.

5. 2. Cochleates and nanocochleates

Cochleates are small lipid-based carriers consist of a negatively charged lipid and a divalent cation. They have a cigar-shaped multilayered structure comprised of continuous, solid, lipid bilayer sheet rolled up in a spiral shape with little or no internal aqueous space. All hydrophobic, amphiphilic, negative or positive charged molecules can be delivered by cochleates and nanocochleates. Also they have been used to deliver peptides, proteins and DNA for vaccine and gene therapy and are able to cover unpleasant taste and smell

of bioactive material intended for oral delivery [22]. Due to their nanometric size, stability and resistance to degradation in the gastrointestinal tract, nanocochleates have revealed great potential to deliver bioactive agents both orally and parenterally. Cochleates containing amphotericin B (AmB) are now in development for the oral and parenteral treatment of fungal infections. The unique structure and properties of cochleates make them an ideal candidate for oral and systemic delivery of sensitive materials including peptide and nucleic acid drugs.

6. CONCLUSIONS

Nanostructured delivery systems are promising candidates enable to an efficient and targeted delivery of novel bioactive compounds. Lipid-based carrier systems, including liposomes and nanoliposomes, are among the most promising encapsulation technologies employed in the rapidly developing field of nanotechnology. Compared with other encapsulation strategies, such as chitosan and alginate-based carriers, lipid based nanoencapsulation systems have some unparalleled advantages, including the ability to entrap material with different solubilities, the feasibility of scale up production and target ability. Several liposome-derived bioactive delivery systems provide a choice of optimized encapsulation and delivery for various applications including systemic and transdermal delivery as well as the choice of short or long-term release. The commercialization of these delivery systems is progressing, as is the development of their preparation methods. Safe and reproducible manufacture of these carriers on industrial scales is now possible. The development of liposomes and their associated products, for pharmaceutical, cosmetics and food industries, continues to be pursued actively by a number of groups globally. Accordingly, it is reasonable to project that this field will experience steady growth for the foreseeable future.

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