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Liposomes in ultrasonic drug and gene delivery ☆

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Abstract

Liposome-based drug and gene delivery systems have potential for significant roles in a variety of therapeutic applications. Recently, liposomes have been used to entrap gas and drugs for ultrasound-controlled drug release and ultrasound-enhanced drug delivery. Echogenic liposomes have been produced by different preparation methods, including lyophilization, pressurization, and biotin–avidin binding. Presently, significant *in vivo* applications of liposomal ultrasound-based drug and gene delivery are being made in cardiac disease, stroke and tumor therapy. Translation of these vehicles into the clinic will require a better understanding of improved physical properties to avoid rapid clearance, as well as of possible side effects, including those of the ultrasound. The aim of this review is to provide orientation for new researchers in the area of ultrasound-enhanced liposome drug and gene delivery.

Keywords: Liposomes; Ultrasound; Drug delivery; Gene delivery; Gases; Nitric oxide

Contents

1. Introduction

The effective delivery of therapeutics to regions of pathology is largely dependent on a sufficient local concentration of a therapeutic agent and an adequate amount of transport of the agent across the endothelial barrier. Liposomal drug delivery

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systems have been investigated for mediating the accumulation of therapeutic agents at specific disease sites in the body [1–4]. Ultrasound-based approaches are now being developed for improving drug and gene delivery by increasing the transport of the therapeutic agent across the cell membrane or endothelial barriers [5–9].

By application of the appropriate parameters, ultrasound can cause cavitation, a process of nucleation, growth, and oscillation of gaseous cavities. Cavitation involves rapid growth and collapse of bubbles (inertial cavitation) or sustained oscillatory motion of bubbles (stable cavitation). Both forms of cavitation can produce strong physical–chemical and biological effects in tissues [5]. In particular, the collapse of cavitation bubbles leads to the formation of shock waves or high-velocity microjets that are capable of disrupting the membrane of cells and tissues and of enhancing drug transport. Because of the scarcity of endogenous gas bodies, blood and tissues normally constitute environments that are recalcitrant to the induction of inertial cavitation unless they are exposed to extremely high US intensities and the associated negative pressures [10]. However, these high intensities carry the risk of producing cell and tissue damage, and thus caution should be exercised. The presence of pre-existent cavitation nuclei, called ultrasound contrast agents, can decrease the threshold for cavitation. In the presence of ultrasound contrast agents, a lower power of ultrasound can facilitate delivery of drugs or genes to a variety of cells such as rat fibroblasts, chondrocytes [11], Chinese hamster ovary cells [12], human cancer cells HeLa, NIH/3T3 C127I [13,14], prostate cancer cells DU145 [7], and various tissues such as muscles [15–18], liver [19,20], lung [19], vasculature [19,21–23], brain, and tumors [8,24–26].

Ultrasound contrast agents usually consist of small gas microbubbles stabilized by a surfactant [27,28]. Currently used surfactants include serum albumin, polymer, and phospholipid [29]. When phospholipid is used as the surfactant for gas stabilization, one of two types of structures is typical: gas bubbles with a lipid monolayer at the surface, or gas-containing liposomes (echogenic liposomes) [30–32]. Echogenic liposomes have the following characteristics when used to enhance ultrasound-facilitated drug and gene delivery.

1) Echogenic liposomes have high drug and gene loading properties similar to those of conventional liposomes.
2) Echogenic liposomes containing entrapped therapeutic agents can be conjugated to antibodies and targeted to specific disease sites, allowing high local concentrations and low systemic toxicity.
3) Release of their contents can be controlled to produce bolus release with a single high amplitude ultrasonic pulse, sustained release by a series of low amplitude pulses, or a combination of the two. These options could be particularly valuable in those cases where it is important to raise the local concentration to a therapeutic level and maintain it for a certain duration of time.
4) Cavitation caused by ultrasound-triggered destruction of gas bubbles increases the permeability of cells and tissues to different sizes of molecules and thus facilitates the transport of the drug or gene into cells and tissues.
5) The ultrasound reflectivity of echogenic liposomes allows image-guided drug and gene delivery.

2. Overview of various echogenic liposomes

Liposomes are self-forming lipid bilayer arrays separating an aqueous internal compartment from the bulk aqueous phase (Fig. 1) [33]. In contrast to lipid monolayer structures, liposomes are characterized by extended, two-dimensional, and clearly separated hydrophilic and hydrophobic regions. The hydrophilic portions of bilayer lipids are directed towards aqueous phases (external and internal), whereas hydrophobic portions of both lipid layers are directed towards one another, forming the internal core of a membrane. A special characteristic of liposomes for drug delivery is that they enable water-soluble and water-insoluble materials to be encapsulated together. Water-soluble materials are entrapped in the aqueous core, while water-insoluble and oil-soluble hydrophobic drugs reside within the lipid bilayer [34]. Since liposomes can be cationic (by including cationic lipids in the formulation), they have the ability to carry large amounts of DNA without special production or handling procedures, and they also produce low immunogenic responses [35,36] during gene delivery. Because of increased stability, improved biodistribution, and optimized circulation in blood, several liposomal formulations have been approved by the FDA [4] (Table 1).

Initially, liposomal preparations were modified to permit gas encapsulation [32,37] in order to be used as a targetable contrast agent for ultrasound imaging enhancement [38–42]. These preparations have been termed echogenic liposomes (Fig. 2 and Table 2). When a gas is encapsulated within a liposome, it can be presumed, for thermodynamic reasons, to behave similarly to hydrophobic drugs in that the gas resides between the two monolayers of the liposome bilayer or perhaps as a monolayer-covered air bubble *within* the aqueous compartment of liposomes [43]. It was found that echogenic liposomes retained the characteristics of conventional liposomes and hence could also be employed to co-encapsulate a gas and either drugs or genes for drug or gene delivery [44–46]. Evidence to date indicates that the stability of gas encapsulation is highly dependent upon the encapsulated gas and the lipid shell properties [47] such as gas diffusion across the lipid shell [48,49], thickness of the lipid shell, size of the microbubble [50,51], and the presence of human serum and albumin [52].

Depending on the preparation methods, three basic echogenic liposomal structures have been developed (Fig. 2 and Table 2). The first is the echogenic liposome with two compartments, as

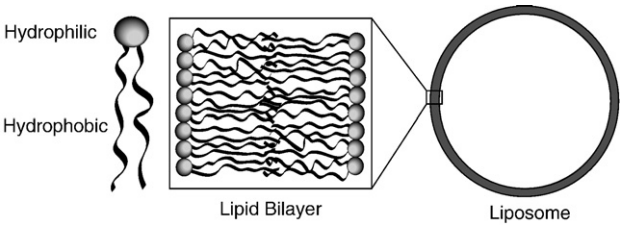

Fig. 1. Schematic diagram of lipids incorporated into a bilayer membrane to form a liposome.