



NANOCOCHLEATE – A NEW APPROACH IN LIPID DRUG DELIVERY

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ABSTRACT

This nanocochleate, able to microencapsulate water-soluble cationic drugs or peptides into its inter lipid bi-layer space, was formed through interaction between negatively charged lipids and drugs or peptides acting as the inter-bi-layer bridges instead of multi-cationic metal ions. This new type of cochleate opened up to form large liposomes when treated with cationic salt, suggesting that cationic organic molecules can be extracted from these cochleates in a way similar to multivalent metal ions from metal ion-bridged cochleates. Cochleates can be produced in sub-micron size using a method known as "hydrogel cochleation" or simply by increasing the ratio of multivalent cationic peptides over negatively charged liposomes. When nanometer-sized cochleates and liposomes containing the same fluorescent labeled lipid component were incubated with human fibroblasts cells under identical conditions, cells exposed to cochleates showed bright fluorescent cell surfaces, whereas those incubated with liposomes did not. This result suggests that cochleates' edges made them fuse with the cell surfaces as compared to edge free liposomes. This ability makes it a potential therapeutic drug carrier.

Keywords: Cationic Drug, Hydrophilic, Hydrophobic, Nanocochleate, Liposomes.

INTRODUCTION

Oral delivery is the most suitable way of administering drugs for most non-hospitalized, non-acute care patients. Drug delivery systems that allow oral delivery improve patient compliance and facilitate treatment outside the hospital, which has a significant impact on healthcare economics. Recently, many drug delivery platforms have emerged and are present in either in preclinical stage or in an advanced clinical trial with the intent of trying to demonstrate efficient oral absorption.¹⁻⁴ According to Egan and Lauri (2002), most of the drugs are absorbed across the tissue membrane by passive diffusion. While particulate therapeutics may be taken up through endocytosis of lymphatic epithelial cells such as Peyer's patches, its efficiency is low because of the limited population of these cells.⁵ Hydrophilic molecules can be absorbed through the water filled tight junctions but only a small portion of drug molecules can have access to the tight junctions along the entire intestinal wall. Although some of the hydrophilic drugs such as nucleosides, anti-folates, and some small peptides are absorbed cross the membrane by attaching to membrane transporters, these transporters are specific to certain therapeutics.^{6,7} Moreover, structural modifications of drug molecules are often required to facilitate receptor-mediated drug molecule absorption, which may alter the pharmacological activity of the drug molecule (Morishita and Peppas, 2006). Therefore, there is a need to develop delivery system, which could facilitate diffusion of the drugs across the cell membrane.⁸

Reported strategies to improve drug absorption through cross membrane diffusion included pro-drug analogue design, application of absorption enhancers and enzyme inhibitors, and delivery by using lipid-based systems⁹. Lipid-based delivery systems including liposomes attracted enormous research efforts as a cross membrane drug delivery vehicle because of their structural resemblance with cell membrane.^{10,11} Utilization of liposomes to improve oral absorption of hydrophilic drugs remains unsuccessful mainly due to their poor mechanical stability, low-drug loading capacity and probably the lack of mechanism to facilitate cross membrane diffusion in intestine.¹² Cochleates are solid particulates made of large continuous, lipid bi-layer sheets rolled up in a spiral structure with no internal aqueous phase. These unique structures were first observed and reported by Papahadjopoulos and Wilschut (1979)¹³ and later by others.

In particular, cochleate technology was shown to be effective in the therapeutic oral delivery of the hydrophobic drugs, which is a negatively charged phospholipids bilayer rolled up by interaction with multi-cationic metal ions to form a rigid spiral rod. It is different from liposome in that it has a water-free interior, a rod-

shape, and a rigid structure. These unique characteristics make cochleates a great platform in delivery of drugs that were not orally bioavailable. Various formulations with liposomes have allowed the development of a new class of delivery vehicles called cochleates. These are stable, lipid based delivery formulations whose structure and properties are very different from liposomes. Liposomes are composed of lipid bilayer membranes with aqueous space bounded by the lipid bilayer.

This lipid bilayer is susceptible to attack by harsh environment conditions like pH, lipase degradation and temperature. Cochleates protect the entrapped molecules from harsh environmental conditions. They are stable to lyophilization and can be reconstituted with liquid prior to administration. Cochleate is most versatile technology for the delivery of a wide range of drugs and fragile molecules such as proteins and peptides. Cochleates are calcium-phospholipid structures composed of naturally occurring materials that "wrap" around the drug or nutrient being introduced into the body. Nanocochleates consist of a purified soy based phospholipid that contains at least about 75% by weight of lipid which can be phosphatidyl serine (PS), dioleoylphosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidyl glycerol (PG) and/or a mixture of one or more of these lipids with other lipids. Additionally or alternatively, the lipid can include phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), dioleoyl phosphatidic acid (DOPA), distearoyl phosphatidylserine (DSPS), and dimyristoyl phosphatidylserine (DMPS), dipalmitoyl phosphatidylglycerol (DPPG). A multivalent cation, which can be Zn²⁺ or Ca²⁺ or Mg²⁺ or Ba²⁺ and a drug, which can be protein, peptide, polynucleotide, antiviral agent, anesthetic, anticancer agent, immunosuppressant, steroidal anti-inflammatory agent, non steroidal anti-inflammatory agents, tranquilizer, nutritional supplement, herbal product, vitamin and/or vasodilatory agent.¹⁴ Thus it is proving to be potential carrier for the wide class of drug for the therapeutics.

DISCOVERY OF NANO COCHLEATES

Cochleates were discovered in 1975 by Dr. D. Papahadjopoulos and co-workers, and have been used in the 80s and 90s to transport antigens and peptides for vaccine delivery. Cochleate structures reported in the literature have not always been uniform, resulting either in aggregates of stacked sheets and cochleates by the trapping method or large size needles-like structures by the Dialysis method.¹⁵⁻¹⁶ Nanocochleates were introduced in 1999 to develop smaller but more consistent particles. It was demonstrated that by using a binary phase system, such as two non-miscible hydrogels, cochleates can be formed that display a small mean particle of less

than 500 nm. These nanocochleates were highly suitable for the encapsulation of hydrophobic drugs.¹⁷

WHAT ARE COCHLEATE

Cochleates are cigar-like microstructures that consist of a series of lipid bilayers (shown in Fig. 1), which are formed as a result of the condensation of small unilamellar negatively charged liposomes. In the presence of calcium, the small phosphatidylserine (PS) liposomes fuse and form large sheets (figure-1). These sheets have hydrophobic surfaces and, in order to minimize their interactions with water, tend to roll-up into the cigar-like cochleate. Freeze-fracture electron microscopy reveals a typical cochleate cylinder characterized by the elongated shape and by the tight packed bilayers.^{18,19}

METHOD OF PREPARATION^{20,21}

Hydrogel method

Formation of the cochleates is multi-step process. In hydrogel method initially the small unilamellar drug loaded liposomes were prepared, which were added to polymer A (Which may be phosphatidyl serine, dextran, polyethylene glycol, etc.). The dispersion of two was then added to another polymer B (which may be polyvinylpyrrolidone, polyvinylalcohol, Ficoll, polyvinyl methyl ether, etc.). The two polymers were immiscible in each other. Immiscibility of the polymers leads to formation of an aqueous two-phase system.

The cationic cross-linking of the polymers was achieved by adding a solution of cation salt to the two-phase system, such that the cation diffuses into second polymer and then into the particles comprised of liposomes/polymer. A allowing the formation of small-sized cochleates. The formed cochleates were then washed to remove polymer, which might be re-suspended into a physiological buffer or any appropriate pharmaceutical vehicle or lyophilized. The schematic diagram is shown in Fig. 2 and its transverse section is shown in Fig.3.

Trapping method

This method involves the formation of phosphatidylserine liposomes followed by dropwise addition of a solution of CaCl₂. Liposomes can be generated by either addition of water to phospholipid powder or by adding the water phase to a phospholipid film.

Liposomes before cochleates(LC) dialysis method

In this method mixture of lipid and detergent were used as the starting material and the removal of detergent was done by double dialysis. The mixture was dialyzed initially with buffer and followed by calcium chloride solutions leads to formation of cochleates. This method was suitable for the encapsulation of hydrophobic material or drugs containing hydrophobic regions such as membrane proteins. In this method intermediate liposome formed were small in size and hence resulted in formation of small cochleates.

Mixture of phosphatidylserine and cholesterol (9:1 wt ratio) in extraction buffer and non-ionic detergent was mixed with a pre-selected concentration of polynucleotide. The resulting solution was vortexed for 5 minutes. The solution was dialyzed overnight using a mixture of dialysate and buffer in ratio 1:200 without divalent cations, followed by three additional changes of buffer leads to the formation of small protein lipid vesicles. The vesicles were converted to a cochleate precipitate, either by the direct addition of Ca²⁺ ions, or by dialysis against two changes of buffer containing 3 mM Ca²⁺ ions, followed by buffer containing 6 mM Ca²⁺.

Direct calcium (DC) dialysis method

Unlike LC method this method does not involve the intermediate liposome formation and the cochleates formed were large in size. The mixture of lipid and detergent was directly dialyzed against calcium chloride solution. In this method a competition between the removal of detergent from the detergent/lipid/drug micelles and the condensation of bilayers by calcium, results in needle shaped large dimensional structures.

Mixture of phosphatidylserine and cholesterol (9:1 wt ratio) in extraction buffer and non-ionic detergent was mixed with a pre-selected concentration of polynucleotide and the solution was vortexed for 5 minutes. The clear, colorless solution which resulted was dialyzed at room temperature against three changes (minimum 4 hours per change) of buffer (2 mM TES N-Tris[hydroxymethyl]-methyl-2 aminoethane sulfonic acid, 2 mM L-histidine, 100 mM NaCl, pH 7.4) containing 3 mM CaCl₂. The final dialysis routinely used is 6 mM Ca²⁺, although 3 mM Ca²⁺ is sufficient and other concentrations may be compatible with cochleate formation. The ratio of dialysate to buffer for each change was a minimum of 1:100. The resulting white calcium-phospholipid precipitates have been termed DC cochleates. When examined by light microscopy, the suspension contains numerous particulate structures up to several microns in diameter, as well as needle-like structures.

Binary aqueous-aqueous emulsion system

In this method small liposomes were formed by either high pH or by film method, and then the liposomes are mixed with a polymer, such as dextran. The dextran/liposome phase is then injected into a second, non-miscible, polymer (i.e. PEG). The calcium was then added and diffused slowly from one phase to another forming nanocochleates, after which the gel is washed out. The nanocochleates proved to promote oral delivery of injectable drugs. By this method the cochleates formed are of particle size less than 1000 nm.

COCHLEATES-CELL INTERACTION^{22,23}

To examine the interaction of cochleates with cell membrane, 2% fluorescent lipid was mixed with polymer(phosphatidyl serine) to form fluorescent liposomes. When cochleates interact with cell membrane involving a fluorescent lipid transfer, cell surfaces become fluorescent under fluorescent microscopes. Fluorescent nanocochleates were prepared by adding drug to the fluorescent labeled liposomes through a charge-controlled cochleation. The fluorescent nanocochleates and fluorescent liposomes were incubated with yeast and HFF cells for 2 h. Then the cells were rinsed with fluorescent-free medium (or water) and observed under a fluorescent microscope. The cells treated with nanocochleates showed a bright fluorescent contour, whereas those treated with liposomes did not.

MECHANISM OF ACTION

The proposed mechanism of the cross membrane delivery of lipophobic drugs loaded in the inter-bi-layer spaces of cochleates is shown in Fig. 4. We hypothesize that inter-bi-layer contents of cochleates are delivered into cells, when lipid bi-layer structure of nanocochleates fuses with the cell membrane and releases the drug.

APPLICATIONS²⁴⁻²⁸

1. Development of a Nanocochleate based ApoA1 Formulation for the Treatment of Atherosclerosis and other Coronary Heart Diseases Hypercholesterolemia, a condition associated with high levels of low-density lipoproteins (LDL), and low levels of high-density lipoproteins (HDL), is universally accepted as a major risk factor for atherosclerosis and other cardiovascular diseases. The inverse relationship between HDLs and heart diseases is well documented. HDL facilitates the cholesterol efflux from peripheral cells and, after enzyme-mediated cholesterol esterification, transports cholesteryl esters to the body.

ApoA1 (a naturally existing lipoprotein) is an important HDL believed to be the most important in enzymatic esterification of cholesterol and then its transport to the liver, thus protecting the vessels against atherosclerosis.

Infusion or intraperitoneal administration of ApoA1 enhances the HDL ability to transport cholesterol to liver and protect against atherosclerosis but the major limitation for the use of ApoA1 as pharmacological/therapeutic agents has been the need for parenteral administration, as ApoA1 is a protein, it is rapidly degraded by GIT enzymes and so it is not delivered to blood as intact molecule.

So nanocochleates can provide a good platform for the delivery of ApoA1 by oral preparations and can bring a revolution in the treatment of atherosclerosis and other heart diseases originating from high blood cholesterol and LDL levels.

2. Biogeode Nanocochleates have the ability to stabilize and protect an extended range of micronutrients and the potential to increase the nutritional value of processed foods.

3. Nanocochleates have been used to deliver proteins, peptides and DNA for vaccine and gene therapy applications.

4. Nanocochleates showed potential to deliver Amphotericin B, a potential antifungal agent, orally and parentally having a good safety profile with reduced cost of treatment. The prepared cochleates of amphotericin B showed improved stability and efficacy at low doses. They showed improved patient compliance.

Delmas et. al. investigated benefits of cochleates containing Amphotericin by using orally administered doses ranging from 0 to 40 mg/kg of body weight/day for 14 days in a murine model of systemic aspergillosis. The administration of oral doses of CAMB (20 and 40 mg/kg/day) resulted in a survival rate of 70% and a

reduction in colony counts of more than 2 logs in lungs, livers, and kidneys. Orally administered CAMB shows promise for the treatment of aspergillosis.

5. Use of cochleates in the delivery of antibacterial agents:

Cochleates would have the advantage of reducing the toxicity and improving the bactericidal activity. For aminoglycosides and linear or cyclic peptides, cochleates should allow oral administration. The proof of principle of the efficacy of anti-TB cochleates was achieved using clofazimine as an antibacterial drug model.

6. Nanocochleates can deliver Omega-3 fatty acids to cakes, muffins, pasta, soups and cookies without altering the product's taste or odor.

7. Bidelivery Sciences International have developed nanocochleates which can be used to deliver nutrients such as vitamins, omega fatty acids more efficiently to cells, and lycopene without affecting the color and taste of food which makes the concept of super foodstuffs a reality, and these are expected to offer many different potential benefits including increased energy, improved cognitive functions, better immune function, and antiaging benefits.



Fig. 1: Cigar like structure with its double fold

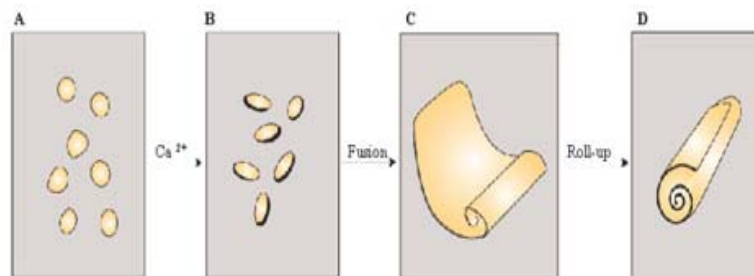


Fig. 2: Cochleate formation from negatively charged vesicles, which fuse after addition of cation to lead to fused liposomes which roll up to give cochleate cylinders

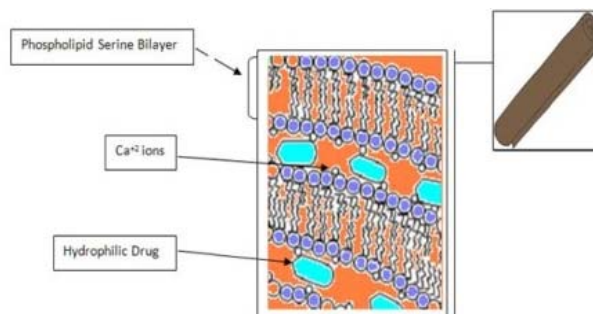


Fig. 3: Transverse section of a rolled cochleate

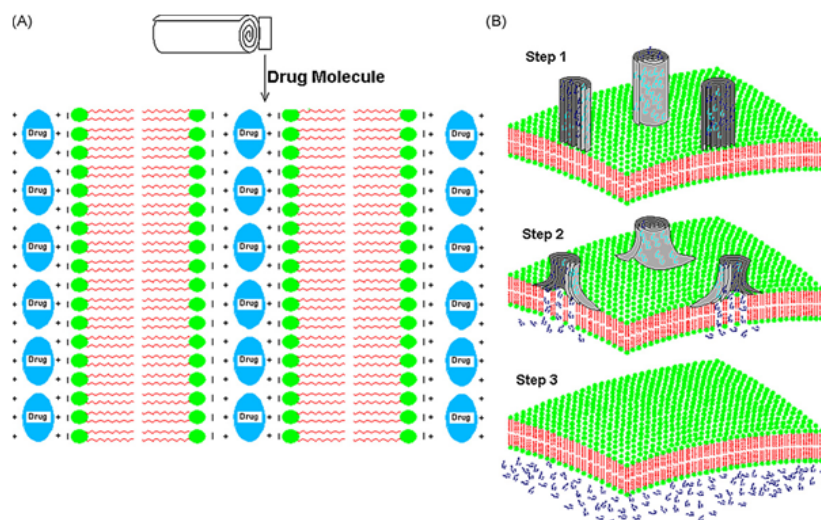


Fig. 4: Diagrammatic presentation of nanocochleate interaction with the cell membrane

CONCLUSION

Since cochleates are formed by ionic interaction between negatively charged liposomes and bivalent cations, microencapsulation of cationic drugs using themselves as the bridging agents of cochleation can be rationalized. Hence this hydrophilic and multicationic drugs and peptides can be successfully encapsulated into cochleates and nanocochleate particles as cochleates' bridging agents. This new type of cochleate is inherent in the physico-chemical properties of metal ion-bridged cochleates in terms of their conversion back to liposomes upon the extraction of cation bridging agent and their ability to fuse with the cell membrane. These unique properties enable cochleates and nanocochleates to deliver charged hydrophilic drugs across the tissue membrane. Thus Nanocochleate possess strong therapeutic potential in the area of drug delivery.

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