NANOCOCHLEATE: AS DRUG DELIVERY VEHICLE

PANWAR VIJETA 2, MAHAJAN VIVEK 1, PANWAR A. S.* 1, DARWHEKAR G. N 1, JAIN D. K. 1

1 COP, IPS ACADEMY, RAJENDRA NAGAR, A. B. ROAD, INDORE (M.P.)

2 N.R.I. INSTITUTE OF PHARMACY BHOPAL (M.P.)

EMAIL: aakash_ips@yahoo.com.sg

ABSTRACT

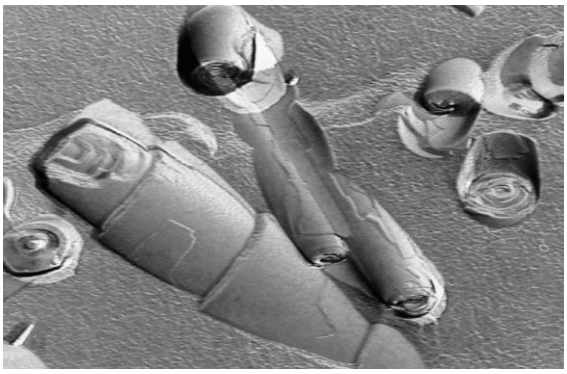
Nanaocochleate represent a new technology for oral and systemic delivery of drugs. It is a novel lipid-based system which represent a unique technology platform suitable for the oral and systemic administration of a wide variety of molecules with important therapeutic biological activities, including drugs, genes, and vaccine antigens. Nanocochleate formulation technology is particularly applicable to macromolecules as well as small molecule drugs that are hydrophobic, positively charged, negatively charged, and that possess poor oral bioavailability. Proof-of-principle studies for cochleate-mediated oral delivery of macromolecules as well as small molecule drugs is being carried out in appropriate animal models with well established, clinically important drugs, which currently can only be effectively delivered by injection.

KEYWORDS: Phospholipid, Liposomes, Phagocytosis.

INTRODUCTION:

The nanocochleate drug delivery vehicle is based upon encapsulating drugs in a multilayered, lipid crystal matrix (a cochleate) to potentially deliver the drug safely and effectively.

Nanocochleates are cylindrical (cigar-like) microstructures that consist of a series of lipid bilayers (Fig.1, 2).[1] Nanocochleate delivery vehicles are stable phospholipid-cation precipitates composed of simple, naturally occurring materials, generally phosphatidylserine and calcium. They have a unique multilayered structure consisting of a solid, lipid bilayer sheet rolled up in a spiral or in stacked sheets, with little or no internal aqueous space. This structure provides protection from degradation for associated "encochleated" molecules. Because the entire nanocochleate structure is a series of solid layers, components encapsulate within the interior of the nanocochleate structure remain intact, even though the outer layers of the nanocochleate may be exposed to harsh environmental conditions or enzymes.[2,3]Because nanocochleates contain both hydrophobic and hydrophilic surfaces, they are suitable to encapsulate both hydrophobic drugs like amphotericin B and clofazimine and amphipathic drugs like doxorubicin.[1]



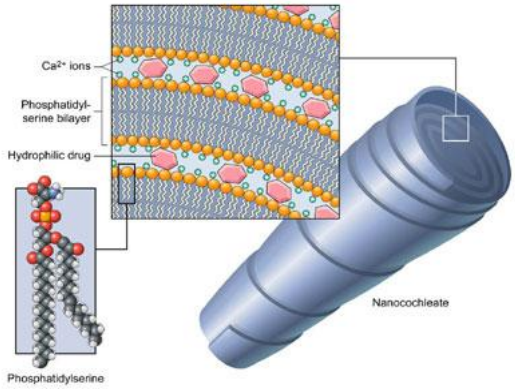


Fig.1: Freeze-fracture electron
Fig. 2: Nanocochleate Structure

Route of Administration

Nanocochleate drug delivery vehicle allows an efficient oral delivery of drugs. An alternative route of administration can be parenteral, rectal, topical, sublingual, mucosal, nasal, opthalmic, subcutaneous, intramuscular, intravenous, transdermal, spinal, intrathecal, intra-articular, intra-arterial, sub-arachniod, bronchial, lymphatic, and intrauterine administration, intravaginal or any other mucosal surfaces.[4]

Dosage form

For oral administration: Capsules, cachets, pills, tablets, lozenges, powders, granules, or as a solution or a suspension or an emulsion.[5]

For topical or transdermal administration: Powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants.[5]

For parenteral administration: Sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use.[5]

Advantages

1. They are more stable than liposomes because the lipids in nanocochleates are less susceptible to oxidation. They maintain structure even after lyophilization, whereas liposome structures are destroyed by lyophilization.

2. They exhibit efficient incorporation of biological molecules, particularly with hydrophobic moieties into the lipid bilayer of the cochleate structure.

3. They have the potential for slow or timed release of the biologic molecule in vivo as nanocochleates slowly unwind or otherwise dissociate.

4. They have a lipid bilayer matrix which serves as a carrier and is composed of simple lipids which are found in animal and plant cell membranes, so that the lipids are non-toxic, non-immunogenic and non-inflammatory.

5. They are produced easily and safely.[6]

6. By the use of nanocochleate IV Drugs to be administered orally (e.g. Amphotericin B, a potent antifungal).

7. They improve oral bioavailability of a broad spectrum of compounds, such as those with poor water solubility, and protein and peptide biopharmaceuticals, which have been difficult to administer. (e.g. ibuprofen for arthritis).[3]

8. They reduce toxicity stomach irritation and other side effects of the encapsulated drug.

9. They encapsulate or entrap the subject drug within a crystal matrix rather than chemically bonding with the drug.

10. They provide protection from degradation to the encochleated drug caused by exposure to adverse environmental conditions such as sunlight, oxygen, water and temperature.[7]

Limitations

1. They require specific storage condition.

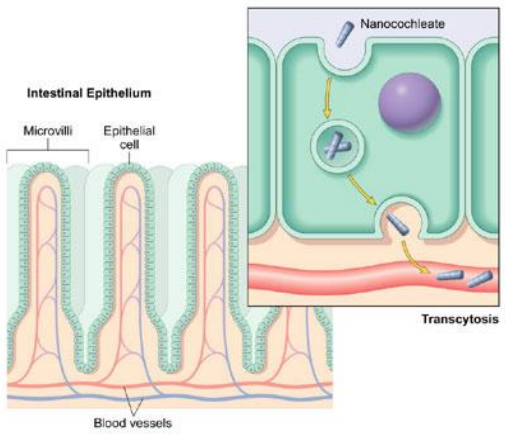
2. Sometimes aggregation may occur during storage; this can be avoided by the use of aggregation inhibitor.

3. The cost of manufacturing is high.[1,6]

Mechanism of Nanocochleate Drug Delivery

Absorption After oral administration nanocochleates Absorption take place from intestine. Nanocochleates cross across the digestive epithelium and deliver their cargo molecules into blood vessel (Fig. 3).[3]

Fig. 3: Nanocochleate Absorption from Intestine



In case of other route except intravenous they cross across the associated cell (in similar manner as discussed above) and reach into circulation.After reaching into circulation they are delivered to targeted cell.[3]

Fig.4: Nanocochleate Delivery to Macrophage

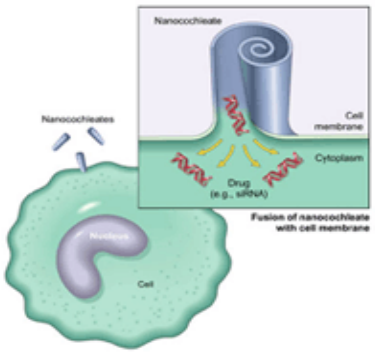
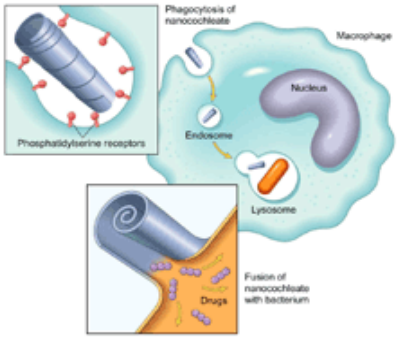


Fig. 5: Direct Membrane Fusion



Delivery to Targeted Cells

The interaction of calcium with negatively charged lipids has been extensively studied. Many naturally occurring membrane fusion events involve the interaction of calcium with negatively charged phospholipids (generally phosphatidylserine or phosphatidylglycerol). Calcium-induced perturbations of membranes containing negatively charged lipids, and the

subsequent membrane fusion events, are important mechanisms in many natural membrane-fusion processes. Hence, cochleates can be envisioned as membrane-fusion intermediates.[3]

1. Delivery after phagocytosis

Macrophages and neutrophils contain membrane phosphatidylserine (PS) receptors which phagocytose nanocochleate.Nanocochleate then comes into close approximation to a liposome membrane, a perturbation and reordering of the liposome membrane is induced, resulting in a fusion event between the outer layer of the nanocochleate and the liposome membrane. This fusion results in the delivery of a small amount of the encochleated material into the cytoplasm of the target cell (Fig. 4).[3]

2. Delivery by cell membrane fusion

In such cases nanocochleate first comes into close approximation to a natural membrane, a perturbation and reordering of the cell membrane is induced, resulting in a fusion event between the outer layer of the nanocochleate and the cell membrane. This fusion results in the delivery of a small amount of the encochleated material into the cytoplasm of the target cell. The nanocochleate may slowly fuse or break free of the cell and be available for another fusion event, either with this or another cell (Fig. 5).[3]

Formulation Methods

Nanocochleates are derived from liposomes which are suspended in an aqueous two-phase polymer solution, enabling the differential partitioning of polar molecule based-structures by phase separation. The liposome-containing two-phase polymer solution, treated with positively charged molecules such as Ca^{++} or Zn^{++}, forms a naocochleate precipitate of a particle size less than one micron. The process may be used to produce nanocochleates containing biologically relevant molecules.[8]

Method 1 (Hydrogel Method): This method comprises of following steps:

Step1- A suspension of small unilamellar liposomes or biologically relevant molecule-loaded liposomes is preparing. This can be achieved by standard methods such as sonication or microfluidization or other related methods.

Step 2- The liposome suspension is mix with polymer A such as dextran (mol wt- 200,000-500,000), Polyethylene glycol (mol wt- 3400-8000) or Phosphatidylserine.

Step3- Preferably by injection, the liposome/Polymer A suspension is added into another polymer B such as poly vinyl pyrolidone, poly vinyl alcohol, ficoll (mol wt- 30,000- 50,000), and poly vinyl methyl ether (PVMB) (mol wt- 60,000- 160,000) in which polymer A is nonmiscible, leading to an aqueous two-phase system of polymers. This can be achieved mechanically by using a syringe pump at an appropriate controlled rate, for example a rate of 0.1 ml/min to 50 ml/min, and preferably at a rate of 1 to 10 ml/min.

Step4- A solution of cation salt is added to the two-phase system of step 3, such that the cation diffuses into polymer B and then into the particles comprised of liposome/polymer A allowing the formation of small-sized cochleates.

Step5- Now to isolate the cochleate structures and to remove the polymer

solution, cochleate precipitates are repeatedly washed with a buffer containing a positively charged molecule, and more preferably, a divalent cation. Addition of a positively charged molecule to the wash buffer ensures that the cochleate structures are maintained throughout the wash step, and that they remain as precipitates.[9]

Method 2(Liposomes before cochleates (LC) dialysis method):

A second method for preparing the small-sized cochleates comprises detergent and a biologically relevant molecule and cation. The detergent is added to disrupt the liposomes. The method comprises the following steps:

Step1- An aqueous suspension containing a detergent-lipid mixture is prepared.

Step2- The detergent-lipid suspension is mixed with polymer A such as dextran (mol wt-200,000-500,000), Polyethylene glycol (mol wt- 3400-8000) or Phosphatidylserine.

Step3- The detergent-lipid/polymer A suspension is added into a solution comprising polymer B such as poly vinyl pyrolidone, poly vinyl alcohol, ficoll (mol wt- 30,000- 50,000), and poly vinyl methyl ether (PVMB) (mol wt- 60,000- 160,000), wherein polymer A and polymer B are immiscible, thereby creating a two-phase polymer system.

Step4- A solution of a cationic moiety is added to the two-phase polymer system.

Step5-Now wash the two-phase polymer system to remove the polymer.[9]

Method 3 (Direct calcium (DC) dialysis method):

Unlike LC method this method dose not involves 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.

Step1- Mixture of phosphatidylserine and cholesterol (9:1 wt ratio) in extraction buffer and non-ionic detergent was mixed with a preselected concentration of polynucleotide and the solution was vortexed for 5 minutes.

Step2- The clear, colorless solution which resulted was dialyzed at room temperature against three changes of buffer

Step3- The final dialysis used is 6 mM Ca2+, and buffer concentrations are maintained compatible to cochleate formation. The resulting white calcium-phospholipid precipitates have been termed DC cochleates.

Method 4 (Trapping method): This method involves the formation of phosphatidylserine liposomes followed by dropwise addition of a solution of Calcium chloride. Liposomes can be generated by either addition of water to phospholipid powder or by adding the water phase to a phospholipid film.

Method 5 (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.

Uses

Nanocochleate is administered to treat at least one disease or disorder selected from the group consisting of inflammation pain, infection, an immune disorder, genetic disorders, cancer, obesity, depression, hypertension, hypotension, schizophrenia, Alzheimer's disease, Parkinson's disease, cell proliferative disorders, blood coagulation disorders, Grave's disease, eczema, hyperlipidemia, hyperglycemia, muscular dystrophy, arthritis, asthma, chronic rhinosinusitis, inflammatory bowel diseases, ulcerative colitis, and Crohn's disease.[7]

Applications

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 highdensity 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 artherosclerosis. Infusion or intraperitoneal administration of ApoA1 enhances the HDL ability to transport cholesterol to lever and protect against atherosclerosis but the major limitation for the use of ApoA1 as pharmacological/therapeutical 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.

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 odour.

7. Biodelivery 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.[8]

Conclusion

Nanocochleate delivery vehicles have been shown to be broadly applicable to a wide range of biologically important molecules. Encochleation can improve an end product by enhancing the qualities of the formulation, increasing processing and shelf-life stability, enhancing bioavailability, reducing toxicity, and increasing efficacy.

Taking advantage of unique properties of Nanocochleates, BDSI has used nanocochleates to mediate and enhance the oral bioavailability of a broad spectrum of important but difficult to formulate biopharmaceuticals, including compounds with poor water solubility, protein and peptide drugs, and large hydrophilic molecules. BioDelivery Sciences and collaborators have performed proof of principle studies in appropriate animal models in each of these areas:

- Nanocochleate-mediated oral delivery of Amphotericin B (Bioral Amphotericin B)
- Nanocochleate-mediated oral delivery of large DNA constructs/plasmids (Bioral DNA Vaccines and Bioral Gene Therapy)
- Nanocochleate-mediated oral delivery of peptide formulations
- Nanocochelate-mediated oral delivery of anti-inflammatory formulations (Bioral Aspirin)
- Autologous HIV Vaccine Development
- Nanocochleate-mediated oral delivery of peptide-based vaccines

These initial animal studies demonstrate that nanocochleate formulations are widely suitable to a broad range of therapeutic applications.

References

1. Zarif, L., & Perlin, D.(2002). Amphotericin B Nanocochleates : From Formulation to Oral Efficacy, Drug Delivery Technology, 2(4), 34-37.

2. Delmarre, D., Lu, R., Tatton, N., Krause-Elsmore, S., Gould-Fogerite, S., & Mannino, R. J. (2004). Formulation of Hydrophobic Drugs Into Cochleate Delivery Vehicles: A Simplified Protocol & Formulation Kit, Drug Delivery Technology, 4(1), 64-69.

3. Biodelivery sciences International (2005). Retrieved September 13, 2006 from http://www.biodeliverysciences.com/AboutNanocochleateTechnology.html

4. Jin, T., Zarif, L., & Mannino, R. (2000). Nanocochleate formulations, process of preparation and method of delivery of pharmaceutical agents, U.S. Patent 6,153,217

5. O'Donnell, Francis E. JR., Gould-Fogerite, S., & Mannino, R. J., Apoprotein cochleate compositions (2006). U.S. Patent Application 2006/0019870 A1

6. Gould-Fogerite, S., & Mannino, R. J. (1997). Cochleate delivery vehicles, U.S. Patent 5,994,318

7. Biodelivery sciences International (2005). Retrieved September 13, 2006 from http://www.biodeliverysciences.com/technology/bioral.html

8. Mannino, R. J., Gould-Fogerite, S., Krause-Elsmore, S. L., Delmarre, D., & Lu, R. (2005). Novel encochleation methods, cochleates and methods of use, U.S. Patent Application 2005/0013854 A1

9. Zarif, L., Jin, T., Segarra, I., Mannino, R. J. (2003). Hydrogel-isolated cochleate formulations, process of preparation and their use for the delivery of biologically relevant molecules, U.S. Patent 6592894