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Review Article

Virosomes: As a Drug Delivery Carrier

Naresh Kalra*, V.Dhanya, Vineeta Saini, Dr. G. Jeyabalan

Department of Pharmaceutics, Alwar Pharmacy College, IET, North extension MIA, Alwar.

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Address for Correspondence
Alwar pharmacy College,North extension, IET MIA alwar (Raj) India

E-mail:
neshu07@gmail.com

ABSTRACT

Virosomes are reconstituted viral envelopes that can serve as vaccines and as vehicles for cellular delivery of various macromolecules. The prospect of drug delivery and targeting systems using virosomes is an interesting research and development field. Since virosomes are biocompatible, biodegradable, non-toxic and non-autoimmunogenuic; attempts have been made to utilize them as vaccines or adjuvants as well as delivery systems for drugs and biological for therapeutic purposes. Influenza virus is the most common virus of choice where virosomes are reconstituted influenza virus envelopes devoid of inner nucleic acid core and hence genetic information. The particulate structure and the function of the surface hemagglutinin protein of binding to the cell receptor, mediates pH-dependent membrane fusion leading to cellular delivery of the encapsulated biologically active molecule. Various protein, peptide and malarial drugs are too loaded into virus to deliver at a particular site to provide targeted drug delivery system.

Keywords: Virosomes, Drug delivery, Genes, Virus

INTRODUCTION

Promising drugs are often discontinued during development because they cannot be suitably delivered to target cells, tissues, and organs. The new generation of therapeutics against cancer or neurodegenerative disorders requires delivery systems that target drugs to specified cell types and host tissues by receptor-mediated uptake and controlled release. Virosomal technology presents a novel sophisticated delivery system to meet these challenges. Virosomes are reconstituted viral envelopes, including membrane lipids and viral spike glycoprotein, but devoid of viral genetic material. The external surface of the virosome resembles that of a virus particle, with spike proteins protruding from the membrane, but their interior compartment is empty. Virosomes were first prepared by Almeida *et al.*, who inserted purified influenza spike proteins into preformed liposomes.[1] Thereafter a range of viral envelopes have been reconstituted, including those of Sendai virus,[2,3,4] Semliki Forest virus (SFV),[5,6] vesicular stomatitis virus

(VSV),[7,8] and Sindbis virus.[9] Because virosomes display viral envelope glycoproteins, which, in their native conformation stimulate humoral responses, they are highly effective as vaccine antigens and adjuvants.[10] Moreover, since the receptor-binding and membrane-fusion properties of the viral envelope glycoprotein can be preserved, virosomes can be used as transport vehicles for cellular delivery of biologically active macromolecules. In this article, we provide a brief overview of virosomal drug delivery.

Overall, virosomes protect pharmaceutically active substances from proteolytic degradation and low pH within endosomes, allowing their contents to remain intact when they reach the cytoplasm. This is a major advantage of virosomal carrier systems over other drug-delivery vehicles, including liposomal and proteoliposomal carrier systems.

Definition

Semi-synthetic complex derived from nucleic-acid free viral particles. They are essentially reconstituted viral coats, where the infectious nucleocapsid is replaced by a compound of choice. Virosomes retain their fusogenic activity and thus deliver the incorporated compound (antigens, drugs, genes) inside the target cell. They can be used for vaccines (VACCINES, VIROSOME), drug delivery, or gene transfer.

Advantages of virosomal drug delivery

- Virosomal technology is approved by the FDA for use in humans, and has a high safety profile
- Virosomes are biodegradable, biocompatible, and non-toxic
- No disease-transmission risk
- No autoimmunogenity or anaphylaxis[11,12]
- Broadly applicable with almost all important drugs (anticancer drugs, proteins, peptides, nucleic acids, antibiotics, fungicides)
- Enables drug delivery into the cytoplasm of target cell
- Promotes fusion activity in the endolysosomal pathway
- Protects drugs against degradation.

Virosome structure

Virosomes are spherical unilamellar vesicles with a mean diameter of around 150 nm. Influenza virus is most commonly used for virosome production. Virosomes cannot replicate but are pure fusion-active vesicles. In contrast to liposomes, virosomes contain functional viral envelope glycoproteins: influenza virus hemagglutinin (HA) and neuraminidase (NA) are intercalated within the phospholipids bilayers membrane. Further characteristics of virosomes depend on the choice of bilayer components. Virosomes can be optimized for maximal incorporation of the drug or for the best physiological effect by modifying the content or type of membrane lipids used. It is even possible to generate carriers for antisense-oligonucleotides or other genetic molecules, depending on whether positively or negatively loaded phospholipids are incorporated into the membrane. Various ligands, such as cytokines, peptides, and monoclonal antibodies (MAbs) can be incorporated into the virosome and displayed on the virosomal surface. Even tumor-specific monoclonal antibody fragments (Fab) can be linked to virosomes to direct the carrier to selected tumor cells.

Difference from Liposomes

Liposomes have been considered promising vehicles for targeting and delivery of biologically active molecules to living cells both *in vitro* and *in vivo*. However, liposomes have little potential to fuse with cells and thus, generally fail to provide appreciable delivery of encapsulated molecules to the cell

cytoplasm. In contrast, virosomes contain functional viral envelope glycoproteins with receptor-binding and membrane-fusion properties that enable the cellular delivery of encapsulated molecules.[13]

Fusion activity of virosomes

Virosomes have unique fusion properties because of the presence of influenza HA in their membranes. HA not only confers structural stability and homogeneity to virosomal formulations, but it also significantly contributes to the fusion activity of virosomes. Virosomal HA promotes binding at the target cell surface followed by receptor-mediated endocytosis. The acidic environment of the endosome triggers HA-mediated membrane fusion, and the therapeutically active substance escapes from the endosome into the cytoplasm of the target cell. Thus, virosomal HA significantly enhances cytosolic delivery. Overall, virosomes protect pharmaceutically active substances from proteolytic degradation and low pH within the endosomes before they reach the cytoplasm. This is a major advantage of the virosomal carrier system over liposomal and proteoliposomal carrier systems, which provide less protection for therapeutic macromolecules from harsh compartmental microenvironments.

Method of preparation

Selection of virosomes

Virosome are reconstituted viral envelope that can be derived from different virosome. Influenza virus envelope is the most commonly used to produce virosome but virosomes can be made from Sendai virus, Epstein burr- virus, HIV, Sindbis, Semlikiforest, virus Friend murine leukemia virus, herpes simplex virus.

Selection of antigen

Antigen is selected as per requirement. Antigen such a parasite, carcinogenic cell, bacterium or whole cell is used. As antigen such as cell component DNA, RNA or plasmid can also used as antigen. This antigen is coupled to lipid anchor so antigen will ready to load on virosomes

Reconstituted of virosome

Virosome solubilised with detergent (octaglucoside, triton x-100, nonidert p-40) Due to solubilization with detergent internal viral protein and genetic material will sediment then detergent is removed by different method such as dialysis and hydrobhobic resins from supernatant. Then using ultracentrifugation process viral matrix protein and nucleicapsid is removed. Viral phospholipid (82%) and viral protein is recovered. Now antigen which is already coupled to lipid anchor is mixed with polymer or surfactant solution and this solution is processed with virosome carrier so that antigen bound virosome is obtained.[14,15,16]

Characterization of virosomes

Protein detection

Virosome preparation should generally result in a relatively uniform protein-to-lipid ratio. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) can confirm the presence of HA protein in the virosomes.[17]

Structure and size

Negative-stain electron microscopy can generally be used to determine the ultra structure and size of virosomes. The staining solutions should preferably be of neutral pH, to avoid acid-induced conformational changes of HA.[18]

Fusion activity

Generally virosomes exhibit pH-dependent membrane fusion activity similar to native influenza virus. Virosomal fusion with biological or artificial target

membranes can be assessed in vitro with an excimer assay using pyrene-labeled lipids, where the decrease of surface density of the pyrene-phosphatidylcholine-label on fusion with an unlabeled membrane corresponds to a reduction of excimer fluorescence.

Fusion activity also can be indirectly monitored by determining hemolytic activity, which corresponds closely to fusion activity and exhibits pH dependence identical with that of fusion

Mechanism of action of virosome

Virosome act both as a carrier and as an adjuvant with multiple functions during the induction of an immune response. The carrier function comprises the positive effects of embedding the antigen into a higher structure, the virosome particle. The adjuvant function relates to immune stimulating properties of virosome and their components on immune system most importantly virosome succeed in stimulating specific immunity without causing non- specific inflammation.

Properties of virosomes

Virosome are biodegradable, biocompatible non- toxic, An antigen can be incorporated into virosome, adsorbed to virosome surface and integrated into to the lipid membrane either hydrophobic domain or lipid moieties cross- linked to antigen. They are also being considered for HIV –I vaccine research. They were used as a drug carrier mechanism for experimental cancer therapies.

Drug delivery approaches

Bioactive drug compounds can be entrapped in the aqueous interior of the virosome or in the lipid membrane of the virosome for facilitated entry of the compounds into the cells.[19]

Virosomes are particularly useful for delivering nucleic acids or genes. These compounds are delivered into the host cell cytoplasm on fusion of the virosome with the endosome or plasma membrane.[20] Nucleic acids or genes encoding a naturally occurring protein can be introduced into host cells and can be expressed, provided that the expression cassette contains the proper cis-acting regulatory elements.[21]

Drugs or nucleic acids can be incorporated into the virosome at the time of virosome preparation. The bioactive compound is typically added to the lipid–HA-containing solution following removal of the nucleocapsid. Alternatively, the bioactive compound is initially incorporated into a liposome, which is then fused with a virosome containing two hemagglutinins with different pH thresholds to form a virosome–liposome hybrid.[22]

Proteins also can be delivered to cells via virosome. For example, the gelonin subunit A of diphtheria toxin and ovalbumin have also been successfully delivered by virosome to target cells.[23] Virosomes carrying peptides derived from the influenza nucleoprotein or intact ovalbumin induced strong cytotoxic T lymphocyte responses, which suggests that the encapsulated peptides and proteins gained access to the cytoplasm.[24,25]

Targeted Drug Delivery

Ideally one would like to be able to target drug delivery to selected tissues. One can tailor virosomes to targets by incorporating specific molecules (e.g., Fab fragments and ligands) into the virosomes composition. The feasibility of targeted delivery of anticancer drugs by means of virosomal carrier has been demonstrated recently by two independent approaches. In one, a MAb cross-linked to the surface of virosomes mediated specific targeting of the virosomal carrier containing an anticancer drug (e.g., doxorubicin) to human cancer

cells. MAbs can bind specifically to cancer-related antigens, providing a means to target systemically administered virosomes to cancerous tissues. Alternatively, ligands that bind surface receptors on the target cells also can be bound to the virosomes to achieve targeted drug delivery. Tumors of mice treated with targeted drug-loaded virosomes failed to grow, and mortality of these animals was significantly reduced. These positive results will definitely open a new field of applications for virosomal technology.

Administration of Virosomes

Several formulations have been reported. Generally, virosomes are suspended in buffered saline (135–150 mM NaCl), but other suitable vehicles also exist. These compositions should be sterilized by conventional liposomal sterilization techniques, such as membrane filtration. The formulation also generally contains auxiliary substances as required to simulate physiological conditions, such as buffering agents and is tonicity adjusting agents (sodium acetate, sodium lactate, sodium chloride, potassium chloride, and calcium chloride). The concentration of virosomes used in the vehicle ranges from 20–200 mg/mL. These concentrations are varied to optimize treatment with different virosome components or for particular purposes.

The virosomes are administered in a variety of parenteral routes, including intravenous, intramuscular, subcutaneous, intra-arterial, and inhalable delivery. In addition, virosomes can be administered topically, orally, or transdermally. The virosomes also can be incorporated into implantable devices for long-term release.

Future Prospects

Virosomes represent an innovative drug-delivery system for various biologically active molecules, but especially nucleic acids or genes, and for numerous indications. The surface of virosomes can be suitably modified to facilitate targeted drug delivery. However, their comprehensive pharmacokinetic profile, bioavailability, clinical effects, and stability should be studied thoroughly to ascertain their long-term reliability as a safe, effective, and affordable means for drug delivery.

Applications of virosome

Cancer treatment

Virosome have been also used in oncology field to carry peptide corresponding to tumour associated antigen as in case of peptide from parathyroid hormone related protein or from recombinant proteins such as her -2 neu Fab combined the anti Fab – doxovirosome combined the anti proliferate properties of the monoclonal antibodies and cyototoxic effect of doxorubicin *in vivo.*

Gene delivery

Haemagglutinin the membrane fusion protein of influenza virus is known to mediate a low P^H dependent fusion reaction between the viral envelope and the limiting membrane of endosomal cell compartment following cellular uptake of virus particle by receptor mediated endocytosis.

RNA\DNA

Small interfering RNA , encapsulated in virosomes, are able to down adequate the synthesis of newly induced and constitulevely expressed protein, overcoming the lack of suitable delivery methods for these molecules Intraperitoneal injection of SiRNA loaded virosome resulted in delivery of nucleotide to cell in peritoneal

Malaria therapy

Virosome represent an innovative

drug delivery system for various biologically active molecules, but especially nucleic acid or genes and for numerous indications. The surface of virosomes can be suitably modified to facilitate targeted drug delivery.

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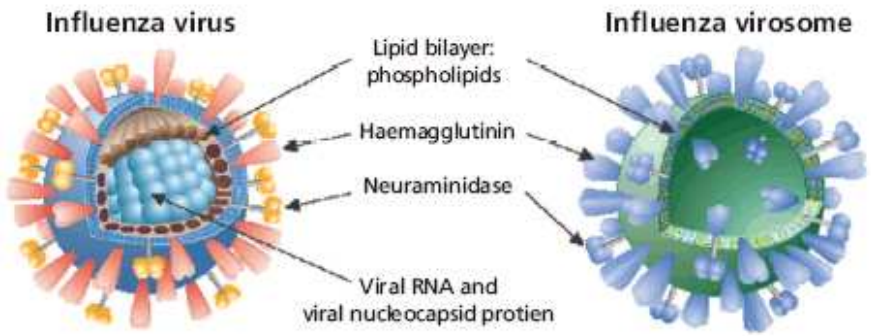

Figure.1. Virus & Virosome