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Review Article

'VIROSOMES' A NOVEL STRATEGY FOR DELIVERY OF DRUGS AND TARGETING: AN OVERVIEW

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ABSTRACT
Over the years there has been a great revolution in drug delivery technologies. Virosomes drug delivery systems are an example of the various novel drug delivery systems available. A virosome is a drug or vaccine delivery mechanism consisting of unilamellar phospholipid bilayer vesicle incorporating virus derived proteins to allow the virosomes to fuse with target cells. Virosomes are not able to replicate but are pure fusion-active vesicle. These are reconstituted viral envelopes that can serve as vaccines and as vehicles for cellular delivery of macromolecules. The prospect of drug delivery and targeting using virosomes is an interesting field of research and development. Because virosomes are biocompatible, biodegradable, nontoxic, and non-autoimmunogenic, attempts have been made to use them as vaccines or adjuvants as well as delivery systems for drugs, nucleic acids, or genes for therapeutic purposes. The success of virosomal drug delivery depends on the methods used to prepare the encapsulated bioactive materials and incorporate them into the virosomes, as are characterization and formulation of the finished preparation. This article gives an insight of virosomes as a newer method of drug delivery. This article gives an insight of hydrogels and virosomes as a newer futuristic tool.
Keywords: Virosome, Biocompatible, Biodegradable, Drug delivery systems, Polymers

INTRODUCTION

A virosome is a drug or vaccine delivery mechanism consisting of unilamellar phospholipid bilayer vesicle incorporating virus derived proteins to allow the virosomes to fuse with target cells. Virosomes are not able to replicate but are pure fusion-active vesicle. These are reconstituted viral envelopes that can serve as vaccines and as vehicles for cellular delivery of macromolecules. The prospect of drug delivery and targeting using virosomes is an interesting field of research and development. Because virosomes are biocompatible, biodegradable, nontoxic, and non-autoimmunogenic, attempts have been made to use them as vaccines or adjuvant as well as delivery systems for drugs, nucleic acids, or genes for therapeutic purposes. Influenza virus is the most common virus of choice. The success of virosomal drug delivery depends on the methods used to prepare the encapsulated bioactive materials and incorporate them into the virosomes, as are characterization and formulation of the finished preparation. Virosomes protect pharmaceutically active substances from proteolytic degradation and low pH within endosomes, allowing their contents to remain intact when they reach the cytoplasm[1].

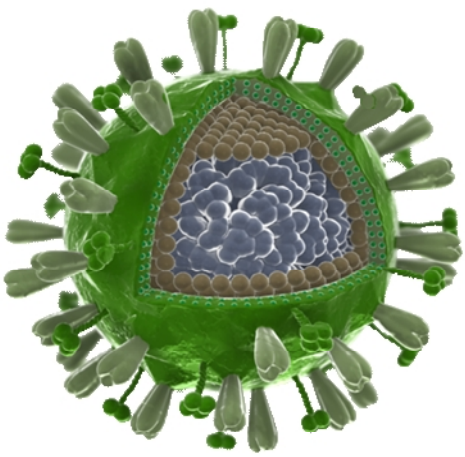
Vehicle for Vaccines[2]

Vaccine is a biological preparation that improves immunity to a particular infectious microorganism (microbe). A vaccine typically contains a small amount of an `A` agent (immunogen) that resembles a microorganism. Upon administration, the immunogen stimulates the body's immune system to recognize it as foreign, destroy it, and "remember" it. Consequently, the immune system can more easily recognize and destroy or neutralize the correspondent microorganism that it later encounters, thereby avoiding severe infection and subsequently, pathologies[3]. The immunogen is made up of some parts of the microbe, as proteins, which are seen by the immune system as the microbe itself. This immunogen usually needs a vehicle to be delivered efficiently in the body.

Structure

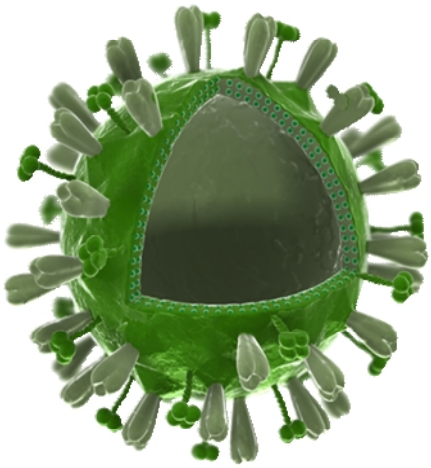
Virosomes are produced by dissolving the envelope of a virus by a detergent or short-chain phospholipid. Next, the viral genetic material and non-membrane proteins are removed. Upon the subsequent removal of the detergent or short-chain phospholipid, the viral membrane is reconstituted, producing a virosome, containing the viral membrane proteins and lipids.Viral proteins confer to virosome based vaccine structural stability, homogeneity and increase the immunologicalproperties of virosomes (fig.1 & fig.2)[4]

Figure-1



As shown in Fig.1, is an influenza virus: the envelope (green) made of influenza lipids constitute the membrane and proteins called Hemagglutinin (HA) and Neuraminidase (NA) are intercalated on it. Inside the envelope the Nucleocapsid (brown) and the genetic material of the source virus (grey).

Figure- 2



As shown in the figure 2, the virus is devoid of the nucleocapsid including the genetic material of the source virus: the virosome is created. Devoid of its substance, it is not able to replicate, cause an infection or a disease.

Virosomes contain functional viral envelope glycoproteins-influenza virus hemagglutinin and neuraminidase intercalated in the phospholipid bilayer membrane[5].They have a typical mean diameter of 150 nm and essentially virosomes represent reconstituted empty influenza virus envelope devoid of the nucleocapsid including the genetic material of the source virus.[6]

Advantages[7]

- 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 auto immunogenity or anaphylaxis.
- 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.
- Target-specific delivery of antigens and amplification of the immune response.
- Extended uptake, distribution and elimination of drug in body.
- Promote fusion activity in endosomal pathway.
- Virosome allow patient specific modular vaccine regimen.
- Up scaling according to standard procedure.
- The fully functional fusion-activity of virosomes enables receptor mediated uptake and natural intracellular of the antigen, which leads to the stimulation of both arms of the immune system such as humoral and cellular immune responses.
- The antigen is partially protected from extra cellular degradation and the resulting depot effect greatly facilitates immune potentiation.

Essentially, virosomes represent empty influenza virus envelopes composed of phospholipids and influenza virus envelope proteins, but they are devoid of internal proteins and genetic material of the parental virus. Virosomes are produced from influenza virus through a detergent solubilization and removal procedure. Properly reconstituted virosomes retain

Adjuvant System[8]

Virosomes are virus-like spherical particles with a mean diameter of 150 nm, consisting of reconstituted influenza virus envelopes, lacking the genetic material of the native virus the cell binding and membrane fusion properties of the native virus, mediated by the viral envelope glycoprotein haemagglutinin These functional characteristics of virosomes form the basis for their enhanced immunogenicity.

Properties[9]

virosomes are biodegradable, biocompatible, and non-toxic. An antigens can be incorporated into virosomes, adsorbed to the virosome surface and integrated in to the lipid membrane,either vie hydrophobic domain or lipid moieties cross-linked to the antigen. They are also being considered for HIV-1 vaccine research. They were used as a drug carrier mechanism for experimental cancer therapies.

Mechanism of Action[10]

Virosomes 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 immunestimulating properties of the virosomes and their components on the immune system. Most importantly, virosomes succeed in stimulating specific immunity without causing nonspecific inflammation.

Carrier Function Response[11]

The integration of the antigen into the higher structure of the virosome particle stabilizes the antigen, preserves the native status of B cell epitopes, and protects the antigen from degradation.The antigen displayed on the virosomal surface mimics the original pathogen or target cell, and thereby favors the generation of antibodies relevant for protection. Moreover, the presentation of the antigen as a repetitive surface structure enhances its recognition by antibody-producing Bcells. Finally, the size and surface structure of the virosome particles make them an attractive target for uptake and processing by immune cells, which is a crucial step in the initiation of an immune response.

Adjuvant Function[12]

The adjuvant function of virosomes relies on the presence of influenza-derived envelope proteins, in particular the predominant haemagglutinin (HA). Pre- existing antibodies against influenza bind to virosomes and tag them efficiently for rapid uptake and processing by antigen presenting cells (APC). The natural function of antibodies is to bind virus and block infection. Also pre-existing influenza-specific helper T cells are activated by those APC displaying the processed fragments of the influenza proteins. Activated helper cells rapidly proliferate and secrete cytokines to support and enhance the induction of effect or immune cells, e.g. antibody-producing cells.

Difference of virosomes from Liposomes[13]

Viral envelope glycoproteins with receptor-binding and membrane-fusion properties that enable the cellular

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 delivery of encapsulated molecules.

PREPARATION OF VIROSOME

a. Selection of virus

Virosome are reconstituted viral envelope that can be derived from different virus. Influenza virus envelope is most often used to produce virosome but virosome can also be made from Sendai virus, Epstein-burr virus, HIV, sindbis, semliki-forest virus, friend murine leukemiavirus, herpes simplex virus, Newcastle disease virus.

b. Selection of antigen

Antigen is selected as per our requirements. Antigens such a parasite, carcinogenic cell, bacterium or whole cell is used as antigens. Cell components such as DNA, RNA or plasmid can also be used as antigen. This antigen is coupled to lipid anchor so antigen will be ready to load on virosome.

c. Reconstitution of virosome

Virosome solubilized 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 hydrophobic resins from supernatant. Then using ultracentrifugation process viral matrix protein and nucleiocapsid is removed. Viral phospholipids (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.

Characterization of Virosomes

a. Protein detection[14]

Virosome preparation should generally result in a relatively uniform protein-to-lipid ratio. Sodium dodecyl sulfate poly15acrylamide gel electrophoresis (SDS-PAGE) can confirm the presence of HA protein in the virosomes.

b. Structure and size[15]

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.

c. Fusion activity[16]

Generally virosomes exhibit pH-dependent membrane fusion activity similar to native influenza virus. Virosomal fusion with biological or artificial target membrane are visualized with a fluorescent resonance energy transfer assay (RET).Alternatively, fusion 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.

Administration of Virosomes[17]

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.

Virosome on Drug targeting[18]

HER-2/neu ($p185^{HER2}$) oncogene represents an attractive target for antibody-mediated immunotherapy. The major problem of combining chemotherapy and immunotherapy is the severe side effects that limit the use of doxorubicin as a cytotoxic drug. We can use virosomes (reconstituted fusion-active viral envelopes) as a new drug delivery system and have shown that virosomes are capable of binding and penetrating into tumor cells, delivering cytotoxic drugs. We have additionally demonstrated that conjugating Fab' fragments of an anti-rNeu monoclonal antibody (mAb) to virosomes selectively and efficiently inhibits tumor progression of established rNeu overexpressing breast tumors.Fab'-Doxo-Virosomes combine the antiproliferative properties of the mAb and the cytotoxic effect of doxorubicin in vivo. Furthermore, Fab'-Doxo-Virosomes significantly inhibit tumor formation at a tumor load representing metastatic spread. These results indicate that virosomes conjugated with an antibody against a tumor antigen are a promising new selective drug delivery system for the treatment of tumors expressing a specific tumor antigen.

Drug-Delivery Approches[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. 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. 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 nucleiocapsid. Alternatively, the bioactive compound is initially incorporated into a liposome, which is then fused with a virosome containing two haemagglutinin with different pH thresholds to form a virosome–liposome hybrid. Proteins also can be delivered to cells via virosome. For example, the gelonin subunit 'A' of diphtheria toxin and ovalbumin has been successfully delivered by virosome to target cells.

APPLICATIONS

Cancer Treatment[20]

Virosomes have been also used in the oncology field to carry peptides corresponding to Tumour Associated Antigens (TAA), as in the case of peptides derived from the parathyroid hormone related protein (PTH-rP) or from the recombinant proteins such as Her-2/neu. Fab'-Doxovirosomes combined the anti-proliferate properties of the monoclonal antibody and the cytotoxiceffect of doxorubicin in vivo. Fab'-Doxo-virosomes significantly inhibited tumour formation at a tumour load of metastasis spread, indicating that this can also be considered to be a promising new selective drug delivery system for the treatment of tumours expressing a specific tumour antigen. The immunogenic effect of the HER-2/neu protein was significantly increased when the protein was linked to virosomes, either encapsulated as a soluble protein or bound to a virosomal membrane. Virosomes elicited humoral and cell-mediated responses against TAA, as well as inducing tumour rejection.

Gene Delivery[21,22]

Haemagglutinin, the membrane fusion protein of influenza virus, is known to mediate a low-pH dependent fusion reaction between the viral envelope and the limiting membrane of the endosomal cell compartment following cellular uptake of the virus particles by receptormediated endocytosis Here, they exploited this activity of haemagglutinin achieve efficient gene delivery to cultured cells[23].

RNA/DNA Delivery[24]

Small interfering RNA (siRNA), encapsulated in virosomes, are able to down regulate the synthesis of newly induced and constitutively expressed proteins, overcoming the lack of suitable delivery methods for these molecules[25]. Intraperitoneal injection of siRNA loaded virosomes resulted in delivery of the nucleotides to cells in the peritoneal cavity.

Murine Lymphocyte activation[26]

The incorporation of Salmonella Minnesota rough- LPS in liposome's reduced the potency of LPS to stimulate splenocyte proliferation and cell surface kappa-light chain expression on 70 Z/3 pre-B cells by over 100-fold[27]. Salmonella Minnesota rough-LPS inserted into virosomes was at least 10-fold more potent than free LPS, both when prebound virosomes were allowed to be taken up by the cells at neutral pH and when the virosomes were fused into the plasma membrane by low pH treatment. Virosomal LPS remained 2- to 10-fold more potent than free LPS in stimulating B lymphocytes and at least 100-fold more active than liposomal LPS or fusion inactivated virosomes[28]. After low pH-induced fusion with the plasma membrane, the majority (80%) of the prebound virosomes had fused with the cells, compared with about 8% after neutral uptake.

Malaria Therapy[29,30]

Malaria vaccine, containing virosome-formulated antimalaria peptides, shows a good tolerability and a highly specific immune response in humans. Scientists have identified and optimized synthetic peptide structures that mimic the surface loops of two candidate malaria vaccine antigens: the NPNA repeat region of the circumsporozoite protein (CSP), loop I of domain III of merozoite apical membrane antigen-1(AMA-1) Lead structures of additional antigens have been identified and additional optimised mimotopes are being developed[30]. It is assumed that there is a need to add further mimotopes to produce an effective multistage, multicomponent malaria vaccine formulation.

Futuristic approach

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 and targeting.

Significant progress has been made in improving the properties of virosomes used for drug delivery and expanding the range of drugs and kinetics which can be achieved using a virosome-based delivery vehicle. However, several challenges remain to improve the clinical applicability of virosomes for drug delivery.

CONCLUSION

Virosome is an emerging system of drug delivery and drug targeting on biological system. Improvisations of this tool will bring a new prospective and also open a new era in the modern pharmaceutical field and also human life too.

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