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VIROSOMES; A NOVAL APPROACH IN NOVAL DRUG DELIVERY SYSTEM: A REVIEW

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ABSTRACT

Promising drugs are often discontinued during development because they cannot be suitably delivered to target cells, tissues and organs. The new generation therapy for various disorders needs a delivery system that target drug to specified cell types and host tissues. Virosomal technology represents a novel sophisticated delivery system to meet these challenges .Virosomes are reconstituted viral envelops that can fill in as vaccines or it can be utilized as vehicles for conveying peptide, nucleic acids and various medications like antitoxic ,anticancer agents and steroids. This safely modified viral envelop mainly consist of a phospholipid membrane and surface glycoprotein. It is derived from several virus envelops .Influenza virosome and sendai virosomes are most common. Their surface can be suitably modified to facilitate targeted drug delivery. How ever their pharmacokinetics clinical effect, bioavailability, stability etc. should be thoroughly studied to ensure long term reliability as safe, effective and affordable means of drug delivery. Thus drug delivery by using biomimetic novel drug delivery systems such as virosomes is a motivating research and development field. This review focus on various features of virosomes such as structure, advantages, disadvantages, formulation, application, kinetics etc

KEYWORDS: Virosome, sendaivirus, Nonimmunogenic, Glycoproteins, heamagluttinin, reconstitution.

INTRODUCTION

The major hurdle for transporting DNA and other biological active substance is crossing the permeability barrier which is made by plasma membrane[1]. To solve this, number of studies and experiments were conducted such as retro viral vectors with cationic liposome and adenoviral based transport system etc, but most of them were failure due to undesirable adverse effects.

Virosomes are reconstituted viral envelops consist of lipid membrane. Viral spike of glycoprotein devoid of viral genetic material having around 15O nm diameter. They are formed by replacement of one or more viral vector by gene of interest. Virus are infectious agents which can replicate in host organism, how everviromsomes do not, due to absence of infectious nucleocapsid. Instead of using live attenuated virus, virosomes utilizes safely killed virus.[2]

Virosomesarebiodegradable,noninfective,biocompatible, nonautoimmunogenic in nature Because of their specific structure and small size they help in transporting biologically active substances. Integration of viral envelop proteins into liposomes were first described by Almeida et.al in 1975.[3] The reconstitution of viral envelops has been applied from several viruses like, -Sendai Virus,-Influenza virus,-Hepatitis B virus,-Semliki forest virus(SFV).

Triumph of virosomal drug delivery depends on various method of preparation, incorporation of gene of choice to viral envelop characteristics, formulation and evaluation of finished product etc.

Virosome for drug targeting system.[4]

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 tumour cells and delivering cytotoxic bioactive drugs. We have additionally demonstrated that conjugating Fab' fragments of an anti-rNeu monoclonal antibody (mAb) to virosomes selectively and efficiently inhibits tumour progression of established rNeu over expressing breast tumours. Fab'-Doxo-Virosomes combine the antiproliferative properties of the mAb and the cytotoxic effect of doxorubicin in vivo. Fab'-Doxo-Virosomes significantly inhibit tumor formation at a tumor load representing metastatic spread. The results indicate that

virosomes conjugated with an antibody against a tumors antigen are a promising new selective drug delivery system for the treatment of tumors expressing a specific tumors antigen.

VIROSOMES

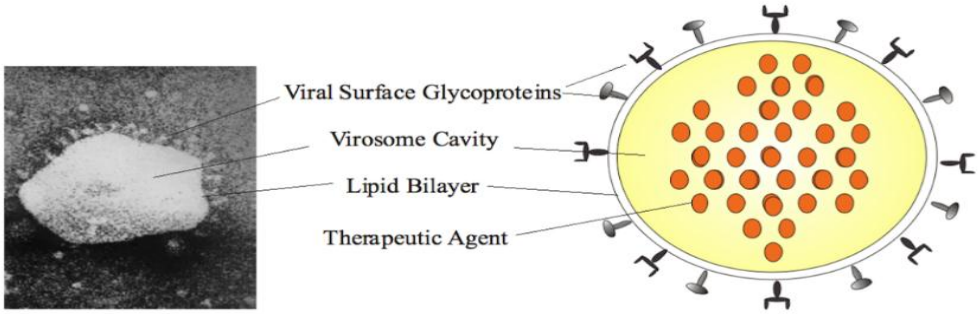

Figure 1: Basic structure of virosomes.

A novel vaccine presentation form that firmly mimic native virus. These are semisynthetic complexnucleicacid devoid of viral particle or viral coats with compound of choice. It act as vehicle which is circular in shape with phosholipid mono/bilayer membrane, inside of it there is a central cavity holds therapeutic molecules such as genes ,protein and drugs. Different types of glycoprotein are present on the surface of virosomes, variability of glycoprotein in surface enhance specificity of target cells because it helps in recognition as well attachment of virosomes to target cells.

Virosome Reconstituted From Influenza Virus

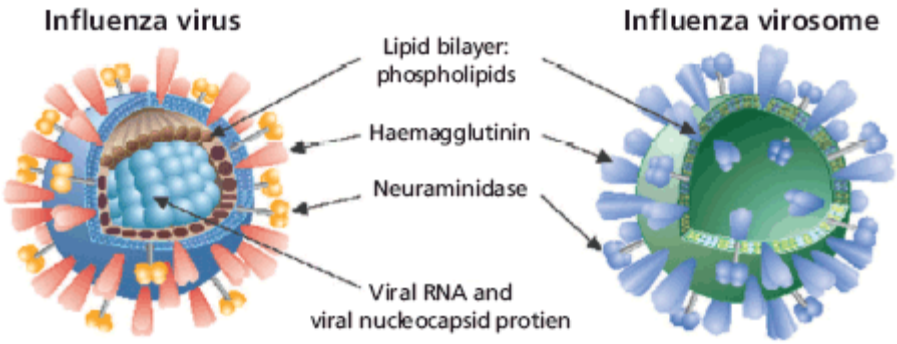

Fig. 2: Virus and Virosome.

Influenza virus is a orthomyxovirus that consist of nucleocapsid with a single stranded RNA genome and covered with a viral envelop.[5,6] Cheif constituent of immuno stimulating regenerate influenza virosome contain naturally occurring phosphatidylcholine 7O% and remaining 3O% of phospholipids. Glycoproteins are inserted inside phospholipid bilayer membrane Neuraminidase (NA) and Heamaglitinin (HA) glycoproteins are present on the surface of the virosomes. HA is responsible for the membrane fusion ability of viral envelop with host cell membrane.[7]unique property due to the presence of biologically active influenza HA in membrane is structural stability and immunological property. HA changes its confirmation at acidic pH to become fusion efficient.NA not only enhance entry of virus into cells but also facilitate release of progeny virus by removing terminal sialic acid from saccharide chain of host cell membrane.

Advantages[8]

- Approved by FDA for human use & has proved to be safe.
- Biodegradable, Biocompatible & nontoxic completely.
- No disease transmission, non autoimmunogenic.
- Delivery of drug into cytoplasm of target cell.
- Protect drug against degradation.
- Enables amalgamated movement in the endolysosomal pathway.
- Administerd either via injection or nasally.
- Up scaling done by possible standard procedure.
- Structures like virus provides repetitive antigen to B cells & mimics natural intracellular performance of the antigen that leads to stimulation of both humoral & cellular immune responses.
- The antigen is protected from extracellular degradation & the resulting depot effect greatly facilitates immune strengthening.

- Enhance controlled uptake, distribution &elimination of drug in the body.
- Good quality& long lasting antibody responses.
- Outstanding safety profile.
- Stabilization of antigen conformation.
- Largely applied with all forms of drugs (Anticancer drugs, Antibiotics & fungicides) and minute organic molecules like proteins, peptides &nucleicacids.
- Suitable for elderly &infants.

Disadvantages[9,10]

- Since they have viral glycoprotein on the surface may induce immune response
- Quick disintegration in the blood compartment.
- Low shelf life.
- Problems during manufacturing.
- Ver poor raw materials quality.
- Pay load is very slow.
- Un availability of data about chronic use of virosome

Applications[11]

Cancer Treatment: Virosomes are used in the oncology field to convey peptides corresponding to tumors associated antigen as in case of peptide from parathyroid hormone related protein or from recombinant protein.sendai virosomes play a major role in anti cancer studies.

Gene Delivery: The membrane fusion protein present in the surface of influenza virus HA, is known to mediate a low pH dependent fusion reaction between the viral envelop and the limiting membrane of endosomal cell compartment followed by the cellular uptake of virus particle by receptor mediated endocytosis.

RNA/DNA Delivery: RNA encapsulated small interfering virosomes are able to down regulate the synthesis of newly induced and constitutively expressed proteins by overcoming the lack of suitable delivering methods for these molecules.

Immune Stimulaio: Virosomes enhance both humoral and cellular immuneresponse virosomes have a pathogen associated molecular pattern (PAMP) that gives stimulatory signals to APC (Antigen presenting cells).

Malarial Therapy: Virosomes containing malarial vaccine is formulated with antimalarial peptides shows excellent tolerability and greater immune responses. Apical membraneantigen-1(AMA-1) and circumsporozoite protein(CSP) are the effectors.

Other Applications

- For making pharmaceutical pigments and dyes.
- Blood substitute for hemoglobin.
- vehicle for drug delivery.
- In dermatology and cosmetics.
- In extraction and separation technique.
- Enzyme immobilization.
- To treat drug overdose.
- For micro and nano capsulated dosage form.
- Anti fungal, antiviral and antimicrobial therapy.

To enhance drug solubilisation.

Route of Administration

Virosomes are administerd in different parenteral routes includes intravenous(IV) intramuscular (IM), Subcutaneous (SC), intraarterial and inhalable etc. Virosomes can also administered via topically, orally, transdermally. It is also formulated as implantable devices for long tern release.

Mechanism of Action of Virosomes[12]

Virosomes act as both as carrier as well as adjuvant during the induction of immune response to our body. The carrier function consist of positive effect of incorporating antigen into a higher structure, virosome. Adjuvent function relates to immune stimulating properties of virosome without causing any non specificinflammation. thus antigen-specific helper immunity induced. There by both humoural and cellular immunity is potentiated.

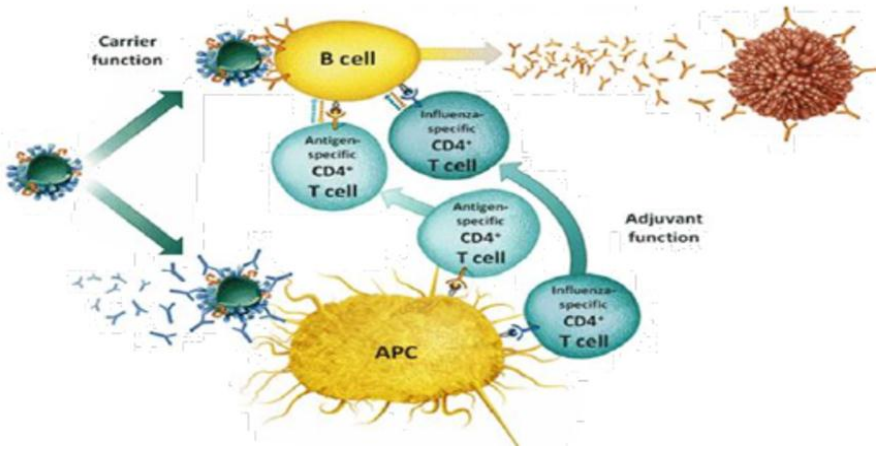

Figure 3: Mode of action of virosomes.

Carrier Functions

Incorporation of antigen into viral envelop stabilizes antigen and preserve status of b cells and protect antigens from degradation. the later mimic original target cell and favors generation of antibody.The presentation of antigen enhance recognition by antibody-producing Bcells.Size and surface nature have crutial role in initiation of immune response.

Properties of Virosomes

Virosomes are biodegradable; biocompatible, and non-toxic. An antigen can be incorporated into virosomes, adsorbed to the virosome surface and integrated in to the lipid membrane, either via hydrophobic domain or lipid moieties cross-linked to the antigen. They are also being considered for HIV-1 vaccine research. Virosomes were used as a drug carrier mechanism for experimental cancer therapies.[13]

METHODS AND DISCUSSION

Method of Preperation of Virosomes[12]

1-selection of virus for virosomes
2-Selection of antigen for virosomes
3-Reconstitution of virosomes

A.selection of virus for virosomes

Virosomes are reconstituted viral envelope devoid of infectious viral nucleus. Influenza virus envelope is most commonly used to produce virosomes but virosomes can also made from Sendai virus, Epstein-burr virus(EBV), HIV, sindbis virus, semliki-forest virus(SFV), friend murine leukaemia virus, herpes simplex virus, Newcastle disease virus etc.[14,15]

B. Selection of antigen for virosomes

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

Reconstitution of Virosomes

Influenza virus (the equivalent of 1.5/tmol membrane phospholipid) is diluted in HNE and sedimented for 30 min at 50,000 g (e.g., in a Beckman TiS0 rotor) at 4°. To the pellet, 0.7 ml of 100 IliA//CI2E8 in HNE is added. The pellet is gently resuspended by repeatedly moving the suspension through a 1-ml syringe with a 25-gauge needle, avoiding the formation of air bubbles. When the pellet is completely resuspended, solubilization is allowed for another 15 min on ice. Based on the assumption that the phospholipid-to-cholesterol molar ratio in the viral membrane is 2: l, the detergent-to-lipid ratio will be approximately 30: 1. Subsequently, the viral nucleocapsid is removed by centrifugation for 30 min at 85,000 g at 4 °. This step is conveniently carried out in a Beckman TL 100 tabletop ultracentrifuge, using 1.3-ml vials plus adapters in the TL100-3 rotor at 50,000 rpm. A small sample of the supernatant can be taken at this stage for protein and phospholipid analysis. Of the initial viral protein and phospholipid, 35% (representing almost all of the membrane protein) and over 90%, respectively, is recovered in the supernatant.

The supernatant (0.63 ml) is transferred to a 1.5-ml Eppendorf vial containing 180 mg (wet weight) pre-washed BioBeads SM2. The supernatant is shaken in a Vibrax-VXR shaker (IKA Labortechnik, Staufen, Germany) at 1400 rpm for 60 min at room temperature. At this stage the suspension is still clear. Subsequently, an additional amount of 90 mg wet BioBeads is added, and shaking is continued for 10 min at 1800 rpm. The suspension becomes turbid at this point, indicating the formation of vesicular structures. Subsequently, the virosome suspension is centrifuged on a 10-40% (w/v) discontinuous sucrose gradient for 90 min at 130,000 g at 4 ° (e.g., in Beckman SW50.1 tubes, containing 1.0 ml of 40% and 3.0 ml of 10% sucrose). The virosomes appear as a thin opalescent band and are collected from the interface in O.5ml.

Antigen loading of virosome[16]

For the encapsulation of antigen in virosomes, the standard virosome procedure as described above can be employed. The desired amount of antigen is added to the C12E8 solubilised viral membrane suspension, before BioBeadtreatment Proteins or peptides can be added in a lyophilised form. We for example added 1 mg of NP peptide, 3–100 mg of ovalbumin (OVA) or 1–5 mg of Human papillomavirus type E7 protein (L. Bunger et al., manuscript in preparation) to 1.5 Amol of viral phospholipid, without any problems concerning the fusion properties of the loaded virosomes.

Reconstitution of other non influnza virosomes

Sendai virus virosomes have been generated by reconstitution of the Sendai fusion protein (F-protein), with or without the hemagglutinin–neuraminidase protein (HN-protein) in viral lipids. Rubella virus virosomes were prepared by incorporating E1 and E2 envelope glycoproteins into liposomes and vesicular stomatitis virus (VSV) virosomes were generated by adding the G-protein of VSV to preformed liposomes. In addition, virosomes have been generated based on Epstein–Barr virus, human immunodeficiency virus, Semliki Forest virus, Friend murine leukemia virus, herpes simplex virus and Newcastle disease virus.

Excipients[16]

Equipped virosomes suspended in saline buffer (135-150 mm NaCl), sterilized by conventional liposomal sterilization method such as membrane filtration. To mimic physiological status they need to contain auxiliary substances such as buffering agents & isotonicity regulating agent like Calcium chloride, Sodium lactate, Sodium acetate, Sodium chloride. The concentration of virosomes used in the vehicle ranges from 20-200

mg/ml, however it can optimized depending on the requirement for the purpose.

Evaluation of virosomes[16]

Protein detection-Relatively uniform protein to lipid⎜ ratio is observed in prepared virosomes. To confirm the presence of HA protein, Sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS- PAGE) method is used.

Surface charge- Free flow electrophoresis

Structure⎜& size- Negative stain electron microscopy with neutral staining agents is used, determined by photo correlation spectroscopy, transmission electron microscopy, dynamic light scattering, and gel permeation & gel exclusion techniques.

Lamellarity- It can be determined 13p-NMR, Freeze⎜ fracture electron microscopy.

Percent free drugs- It can be determined by mini⎜ column centrifugation, gel exclusion, radiolabel ling, protamine aggregation.

Phase behaviour- Differential scanning colorimetry,⎜ freeze fracture electron microscopy.

Drug release- Diffusion cell dialysis

Animal toxicity- It can be determined by monitoring⎜ history, pathology & survival rates.

Pyrogenicity- It can be determined by Rabbit fever test,⎜ Limulus ambeocyte lysate (LAL) test.

Surface chemical analysis- Static secondary ion mass⎜ spectrometry.

Pharmacokinetics of Virosomes[16]

Information regarding differences in the pharmacological effect of free drug & encapsulated drug can be given by the pharmacokinetic studies that help in dose designing. it can give the information regarding the absorption, distribution & degradation time course of retention, dissemination & debasement of the virosomal transport in-vivo. Effect of pharmacokinetic parameter on virosomes shown following outcomes.

- Greater therapeutic index.
- Greater concentration at targeted sites
- Protection of drug in plasma
- Decrease in toxicity & nonspecific reaction.
- Reduction in nonspecific localization.

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. Virosomes can be used to deliver an antigen in the administration by various routes like intranasal, intradermal, and intramuscular,topically, orally -depending on the aim of immunization without any adverse effects.Virosomes can be used to treat various cancers and neurodegenerative disorders. An attractive feature of virosomes is the possibility to target them to selected cells using Fab fragments of monoclonal antibodies specific for the binding. Reconstituted influenza virosomes can be a great tool for delivering antigens and molecules of different nature, such as proteins, peptides, plasmids, oligonucleoides and even drugs to cells. Also other virosomes are obtained like sendai virosome, epstein burr virosomes, etc.and it is possibie to exploit them in different scientific sectors. Cancers are heterogeneous and can transform themselves to be resistant to the treatment that they have received and to escape from the environment of cancer treatment. In this scientific research field, it is absolutely necessary to identify the genes that direct tumorigenesis. However, in the clinical field, it is very important to prepare cancer treatments using a variety of therapeutic principles. Clinicians should provide cancer patients with the appropriate therapeutic tools according to the patient's condition.

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