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Review on virosomes

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Abstract

Virosomes are reconstituted viral envelopes that can fill in as vaccines and as vehicles for cell conveyance of different macromolecule .The prospect of drugs delivery and targeting system utilizing virosomes it's an intriguing innovative work field. Since virosomes are biocompatible, biodegradable, non-poisonous and non-auto immunogenic; endeavours have been made to use them as antibodies or adjuvants and also conveyance framework for drugs and organic for remedial purposes. The achievement of virosomal medicate conveyance relies on upon strategy used to set up the typified bioactive materials and fuse them into the virosomes. Virosomes innovation could conceivably be utilize to convey peptides, nucleic acids or, then again qualities and medication like anti-toxins, anticancer agents, and steroids.

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Introduction

Virosomes are spherical, unilamellar vesicles reconstituted of viral envelops phospholipids with removed nucleocapsid. Virosomes are covered with viral envelops, lipid membrane, viral spike glycoprotien but free from viral genetic material. Virosomes were first prepared by almeida et al., who inserted purified influenza spike protein to liposomes [1]. The external surface of virosomes resembles that virus particals with spike protein from the molecule membrane [2]. The internal surface of virosomal compartment is fully empty. The new invention of therapeutic against cancer or neurodegenarative disorders involves delivery system facilitate target drug toward the host cell or tissues. After that viral envelops their constituted, including those SENDAI VIRUS, SEMLIKI FOREST VIRUS, VESICULAR STOMATIS VIRUS and SINDBIS VIRUS [2]. Virosomes shows viral envelop of glycoprotein and their native conformation stimulates humoral or hormone responses. SENDAI VIRUS virosomes used to generate by reconstitution of SENDAI FUSION PROTEIN [1].

These virus are highly effective as vaccine antigens and adjuvants. Virosomes can be preserved from receptor – binding, membrane-fusion properties of the viral envelopes glycoprotein. Virosomes are mainly used to transport vehicles for Cellular delivery of biologically active macromolecules virosomes comes under, it is mainly developed in combining agents like antigens , drugs etc. In virosomes have the advantages, it is virosome protect the pharmaceutically active ingredient from proteolytic degradation and low PH with in endosomes [4].

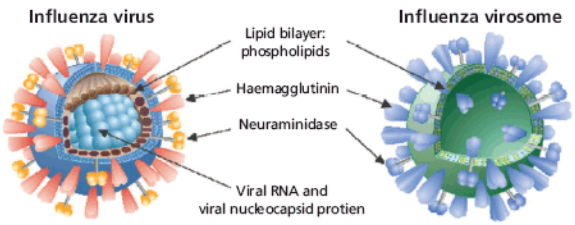

Fig. 1 Structure of Virosomes

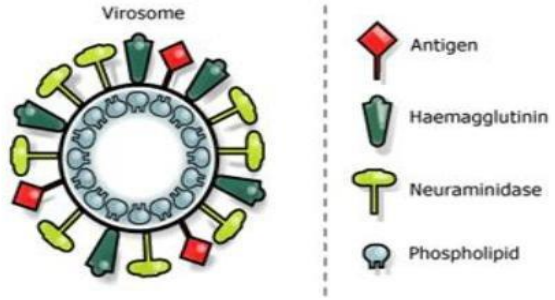

Fig. 2 diagram represents different compartments of virosomes

Fusion 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 cell3. 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 compartment microenvironments [2].

Advantages of Virosomes

1. Virosomes are biodegradable, biocompatible and non toxic.
2. No disease – transmission risk, no auto immunogenicity [1].
3. This technology is approved by FDA for use in humans and has proven to have safety profile [FDA-food and drug administration [1].
4. Broadly applicable with almost all important drugs. Like – anticancer drugs, proteins, peptides, antibiotics ect [2].
5. Promotes fusion activity in the endolysosomal pathway.
6. Protect drugs against degradation [3].
7. Enables drug delivery into the cytoplasma of target cell [1].

Disadvantages of Virosomes

1. Short shelf life [2].
2. They might include immune responses since they often have viral glycoprotein on the surface [1].
3. Pay load is too slow [3].
4. Manufacturing problems.
5. Poor quality of raw material.
6. Un availability of data about chronic use of virosomes [6].

PROPERTIES OF VIROSOMES

Virosomes are biodegradable, biocompatible non-toxic, an antigen can be incorporated into virosomes5. Absorbed to virosomes surface and integrated into the lipid membrane or moieties cross-linked to antigens. They are also being considered for HIV-1 vaccine research. They are used as a drug carrier mechanism for experimental cancer therapies [3].

Methods of Preparation of Virosomes

1. Selection of virosomes.
2. Selection of antigens.
3. Reconstitution of virosomes.

Selection of Virosomes

Virosomes are reconstitution viral envelop that can be derived from different Virosomes. Influenza virus envelops is most commonly used to produce Virosomes but virosomes can be made from other types of viruses also such as SENDAI VIRUS, EPSTEIN – BURR VIRUS, HIV, SINDBIS VIRUS, SEMLIKI FOREST VIRUS , FRIEND MURINE LEUKAEMIAVIRUS , HERPES SIMPLEX VIRUS , NEW CASTEL DISEASE VIRUS [1,2].

2. SELECTION OF ANTIGENS.

Antigens have a some cell compartments like DNA, RNA, and PLASMID can also as antigens [1]. Antigens is selected as per necessary Antigens which are used like bacterial parasite, carcinogenic cell, bacterium or whole cells [3]. This type of antigens are coupled to lipid anchor so antigen will ready to load on virosomes.

3. RECONSTITUTION OF VIROSOMES

Virosomes solubilised with detergents like OCTAGLUCOSIDES, TRITON X-100, NON IDERT P401. Due to the solubilization Of virosomes with detergent internal viral protein and genetic material will be rest then detergent is removed by dissimilar method like dialysis and hydrophobic mesin from supernant3. Then using ultracentrifugation process spike protein and Necleicapsid is removed. Phospholipid [82%] and viral protein is recovered. Now antigens which is already coupled to lipid anchor is mixed with polymer and this polymer is processed are bound virosomes is obtained [4].

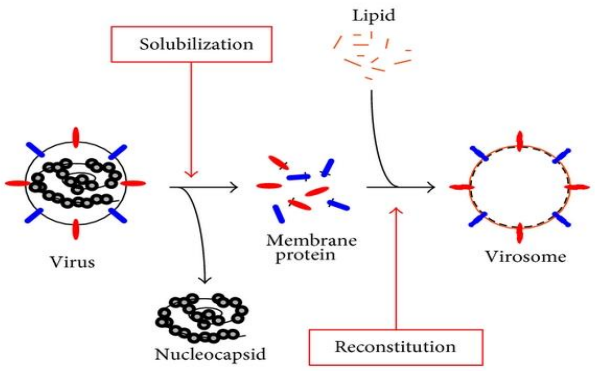

Fig. 3 The schematic representation of current methods of preparation of virosomes.

Mechanism of Action

Virosomes act antigen and antibodies as a carrier, virosomes can act as immunopotentiating agents and as agents of targeted drug delivery. Virosomes as immunopotentiating agents activate cell mediated and humoral immune response1. The carrier function of comprises the positive effects of embedding the antigen into a higher structure, the virosomes particle. The adjuvant function relates to the immune stimulating properties of virosomes and their components on immune system most importantly virosomes succeed in stimulating specific immunity without causing non-specific inflammation [2, 3].

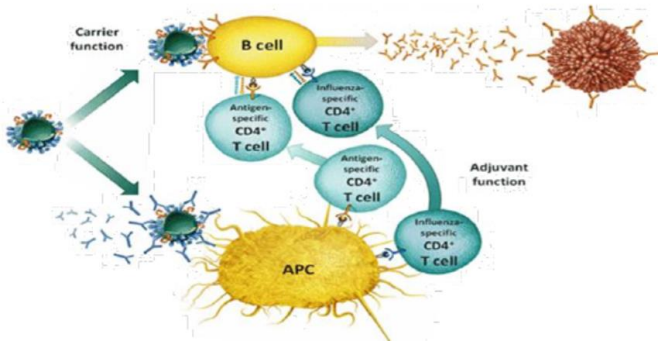

Fig.4 Mechanism of Action of virosomes

Drug Delivery Approach

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. Virosomes are particularly useful for delivering nucleic acids or genes. 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 elements1. 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. Proteins also can be delivered to cells via virosomes2. For example, the gelonin subunit A of diphtheria toxin and ovalbumin have also been successfully delivered by virosome to target cells. Virosomes carrying peptides derived from the influenza nucleoprotein or intact ovalbumin induced strong cytotoxic T lymphocyte responses, which suggests that the Drug Deliverencapsulated peptides and proteins gained access to the cytoplasm [1, 2].

Charecterization of Virosomes.

In virosomes characterized by three types.

1. Detection of protein.
2. Fusion activity.
3. Structure and size.

1 .Detection Of Protein

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 virosomes5.

2. Fusion Activity

Virosomes exhibit PH dependent membrane fusion activity similar to native influenza virus and can be visualised with a fluorescent resonance energy transfer assay [RET]. Fusion activity can also be monitored indirectly by determining haemolytic activity, which is indirectly corresponds to fusion activity and shows PH dependence similar to that of fusion3.

3. 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 HA4.

Targeted Drug Delivery

Virosomes are immune modulating liposomes consisting of surface glycoprotein of influenza virus muramyl dipeptide etc. virosomes must be target oriented and their furogenic characteristic could be exploited in genome grafting and cellular micro injection1. Ideally one would like to be able to target drug delivery to selected tissues one can tailor virosomes to target by incorporating specific molecules [eg. Fab fragments and ligands] into the virosomes composition [8]. The possibility of targeted delivery of anticancer drugs by means of virosomes carrier has been displaced recently by two independent approaches. Monoclonal antibodies MAbs can bind specifically to cancer related antigens, providing a means to target systemically administered virosomes to

cancerous tissues3. Tumors of mice treated with targeted drug located virosomes failed to grow, mortability of these animals was significantly reduced8. These positive results with definitely open a new field of application for virosomal technology. 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 [1].

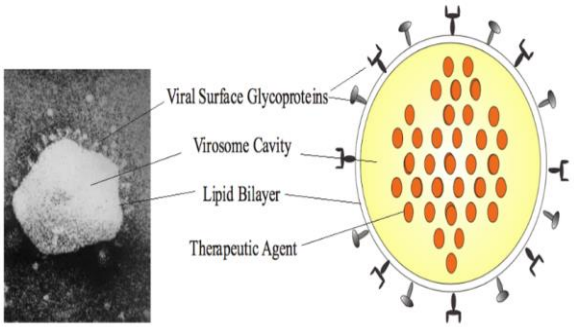

Fig. 4 Composition of virosomes

Virosomes Uptake by Cells

Entry of virosomes into target cells divided into four types

[a] Attachment

This includes official of the virosomes by means of HA to the cell receptors that are a film glycoprotein or glycolipid with terminal sialic corrosive. If there should be an occurrence of particular virosomes, Fab sections are coupled by a cross-linker with a spacer arm to the virosomal surface. Particular virosomes will also perceive antigenic structures on the focusing on cell surface and bringing about a connection to target cells by two distinctive restricting components. Along these lines, particular virosomes apply selectivity for extraordinary cell sorts [3].

[b] Penetraction

After Penetration entry of virosomes happens by receptor-intervened endocytosis. The virosomes are caught in endosomes, acidic combination of the virosomal film with endosomal layer. The combination is interceded by the viral spike glycoprotein hemagglutinin (HA). The layer combination response in the endosome librates the virosomes from its lipid envelope and gives access to the embodied medications to the cytosol [2].

[c] Function of the Carier

The reconciliation of the antigen into the higher structures of the virosomes molecule balance out the antigens, safeguards the local status of B cell epitopes and shields the antigens from debasement. In addition, the introduction of the antigen as a monotonous surface structure improves its acknowledgment by counter acting agent creating B cells [5].

[d] Memory Support

The nearness of flu inferred hemagglutinin (HA) incites a memory reaction as a greater part of individuals have a level of common, prior insusceptibility against flu. This contains both humeral and cell invulnerability: previous flu particular Antibodies tag virosomes proficiently for fast take-up and handling by antigen introducing cells (APC). Memory T partner cells rapidly multiply besides discharge cytokines to help and upgrades Target-specific delivery of antigens and amplification of the immune response5.

Pharmacokinetics of Virosomes

Pharmacokinetics of virosomes regarding difference in the pharmacological effects of free drug and encapsulated drug can be given studies that help in dose designing [2]. It can give the information regarding absorbtion, distribution and degradation time course of retention, dissemination and debasement of the virosomal transport in-vivo [4].

Some pharmacokinetics parameter should be effect by virosomes.

- Greater therapeutic index [1].
- Greater the concentration at targeted site [3].
- Decrease in toxicity and non-specific reaction [6].
- Production of drug in plasma [4].
- Reduction in non-specific localization [2].

Evaluation of Virosomes

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 [6].

- 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 [2].
- Phase behaviour- Differential scanning colorimetry, freeze fracture electron microscopy5.
- 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 [3].
- Surface chemical analysis- Static secondary ion mass spectrometry.

Adminitration of Virosomes

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 [2].

Several formulations have been reported. Generally, virosomes are suspended in buffered saline (135–150 mM NaCl), but other suitable vehicles also exist5. 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 purposes3.

Applications of Virosomes

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 [2].

Gene delivery

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 endosomal cell compartment following cellular uptake of virus particle by receptor mediated endocytosis [3].

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 [1,3].

Maleria Therapy

Virosomes shows good tolerability & highly specific immune responses it has been identified two peptides structures serving as antigens for malaria vaccines. The NPNA regions of circumsporozoite protein (CSP) loop I of domain III of merozoites apical membrane antigen-1 (AMA-1) that leads additional structural antigens that identified

- Immune stimulation – Virosomes provides pathogenassociated molecular pattern (PAMP) that that gives stimulatory signals to APC4
- Virosomes containing antibacterial, antimalarial, antifungal have shown effective Invitro & in vivo profile, the virosomes based sedates conveyance is in any case, quick, sheltered & viable rather than other related system.
- Apart from this virosomes can also delivers proteins & peptides eg- gelonin subunit A of diphtheria toxin has been delivered to target cells by virosomes as well as ovalbumin. Cancer treatment – Cancer is one of the major health [3].

Conclusion

Virosomes represents an innovative novel drug delivery system for various biologically active molecules including nucleic acid & genes for different purpose, they are safe, completely biocompatible, and biodegradable their surface can be modified to facilitate targeted drug delivery. They can be delivered to the host body through altered routes like intranasal, intradermal, and intramuscular depending on the aim of immunization. Tissue targetting immune activation & potentiation are the chief advantages that making them efficient prophylactic & therapeutic agent, they can be exploited as carrier for targeted drugs & for immunomodulating molecules particularly in cancer therapy, Influenza virus considered as promising model for antigen & unrelated molecules delivery that could be helpful for development of new vaccines. Application of Virosome in drug delivery will bring a new prospective & also open a new era in the modern pharmaceutical field & also human life too.

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