

Bilosomes Based Drug Delivery System

Keywords: Bilosomes; Vesicular drug delivery; Bile salt, Hepatitis B; Oral immunisation

Abstract

Vesicular carrier systems (liposomes & niosomes) are one of the potential candidates for vaccine delivery by the oral route. But its instability in gastrointestinal environment makes it less applicable to be used for the purpose of oral immunization. This necessitates larger and more frequent doses of antigen for vaccination. Drug delivery system overcomes which a lipid vesicle is containing bile salts (bilosome), which prevents antigen degradation and enhances mucosal penetration. New generation bilosomes are more stable in gastrointestinal tract. This review is sharply focused on comparative overview of liposomes, niosomes and bilosomes. Bilosomes benefits, future perspective, market application characterization and stability of bilosomes.

Introduction

Whilst the prophylactic intramuscular vaccines used to prevent the spread of influenza have been available for 50 years, there is a substantial degree of vaccination failure amongst the elderly [1,2]. Only 30-70% of people aged 65 years or older are successfully immunised against influenza in any one season, compared with a success rate of 70-80% in younger and healthier individuals [1,2]. The problem is further compounded by the fact that more than 40% of high risk individuals do not receive this vaccine [3]. Therefore, it can be concluded that a vaccine with high efficiency together with the ability to increase patient compliance, would reduce the high death rate associated with this seemingly innocuous virus.

Within the past decade, a number of studies on developing influenza vaccines have been carried out, with most researchers investigating parenteral or nasal immunisation strategies. However, despite the recent shift of attention towards intranasal administration and oral vaccination is still considered the best approach to increase patient compliance. Several oral delivery systems have been extensively studied, including liposomes and the recent innovation, the bilosome [4].

Bilosomes consist of deoxycholic acid incorporated into the membrane of niosomes [4]. Bile salts are commonly used as penetration enhancers by the pharmaceutical industry and promote oral bio-availability [3,5]. In the past, larger and more frequent oral antigen doses were given to overcome the digestive barrier [3,6]. This in turn led to problems such as the induction of oral tolerance. Another advantage of the bilosome is they are produced from naturally occurring lipids making them biocompatible. The bilosome components have also been shown to possess inherent adjuvant properties when associated with antigen [4].

What are Bilosomes

Bilosomes are specialised delivery vehicles which protect vaccines from being broken down in the stomach, thereby enabling the oral



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delivery of vaccines as an alternative to administering treatment by injection.

In 2004, Mann et al., developed non-ionic surfactant vesicle (NISV) having liposome like structures and stabilized them with bile salts for the oral delivery of vaccines. These were called bilosome, bilosomes differ from the liposomes and niosomes in term of their composition, chemical stability and storage conditions (Table 1). In order to avoid the problems during GI transit, bilosomes were developed which not only prevented antigens from degradation, but also enhanced mucosal penetration. Bilosome based vaccine produced both systemic as well as mucosal immune response which was equivalent to immune response produced by subcutaneous route [7].

Table 1: Comparative overview of liposomes, niosomes and bilosomes.

Parameter	Liposomes	Niosomes	Bilosomes
Composition	Natural Phospholipids, cholesterol	Non-ionic surfactant with cholesterol	Non-ionic surfactant and bile salt
Chemical stability	Phospholipids undergo the oxidative degradation	Stable	Stable
GIT stability	Unstable	Unstable	Stable
Antigen dose	Comparatively high	Comparatively high	Comparatively low
Storage and handling condition	Required special conditions (liquid nitrogen storage)	Do not required special conditions	Do not required special conditions

Two years later, new bilosome based vaccine formulation i.e. tetanus toxoid using vesicles containing bile salts for oral delivery were studied [8]. Nanotoxicity and adjuvant effect of NISV were established [9]. The ability of orally administered bilosome based vaccines to induce systemic immune responses in mice, using a standard antigen (bovine serum albumin), a synthetic measles peptide and an influenza sub-unit vaccine were studied. Immune response of above vaccine formulations was increased by incorporating bile salts (in particular deoxycholate). Bilosome based measles peptide vaccine stimulated a specific cell mediated response. Orally administered bilosome based influenza sub-unit vaccine induced systemic immune response same as that produced by parenterally administered vaccine containing the same quantity of antigen [10]. Bilosomes containing bovine serum albumin (BSA) as a model antigen and its conjugate with cholera toxin B subunit (CTB) were prepared. Immune response produced by a single oral dose of cholera toxin B (CTB) conjugated bilosome was almost equivalent to immune response, produced by parenteral administration of antigen with Freund's complete adjuvant (FCA).

Despite this similarity in the immune response, bilosomes has advantages like oral administration is convenient and devoid of any adverse effects as compare to parenteral administration of FCA [11]. HBsAg loaded bilosomes, when given orally, produced high systemic as well as mucosal immune response. Orally administered five times dose of HBsAg loaded bilosomes was found to produce same systemic immune response as that of intra-muscularly administrated alum adsorbed HBsAg vaccine [12]. Other studies carried out using bilosomes are listed in (Table 2).

Benefits

- Bilosomes allow small quantities of antigen to be effective and also increase the efficacy of antigen which are weak when injected.

- Bilosomes do not require the use of live pathogens, making them a safe and effective alternative to traditional vaccines.
- This non-invasive system offers advantages in terms of user acceptance and compliance.
- The conventional injection method suffers from high relative costs and requires trained persons to administer the treatment.
- Less toxicity envelope suitable for a wide range of therapeutic agents.
- Immune response could be manipulated through control of the size of the carrying vesicle.
- Bilosomes removes the cold-chain requirement for preparations such as vaccine.
- Bilosomes provides new delivery system improving patient compliance, its ease of administration and potentially providing extended patent life.

The bilosomes patent represents a major step forward in vaccine technology by avoiding common problems associated with injections and increasing the efficacy of vaccines.

Bilosomes using Oral Immunization against Hepatitis B Virus

Hepatitis B is a type of stern and deadly viral disease caused by hepatitis B virus (HBV). It is a partially double-stranded DNA virus of the Hepadnaviridae family which includes related viruses responsible for liver injury in animals [15]. HBV is carried in blood and in other body fluids including tears, saliva, semen and vaginal secretions and can be transmitted from person to person by a variety of means.

In some studies it was concluded that after its acute infection,

Table 2: Bilosomes based vaccine formulations.

Bilosomes Composition/Type	Vaccine/Antigen	ROA	Inference	Ref.
1-Monopalmitoyl glycerol, cholesterol and dicetyl phosphate, deoxycholic acid	Influenza vaccine	Oral	Significant IgG1 titres were induced when the protein loading was doubled from 15 to 30 µg and was equivalent to antigen administration by the subcutaneous route. No IgG2a was induced, indicating the generation of a Th2 response. Significant mucosal IgA levels were also observed	[7]
Non-ionic surfactant vesicles, deoxycholic Acid	Tetanus Toxoid vaccine	Oral	Entrapped TT, caused a rise in the numbers of IgA positive plasma cells observed in the first 6 in of the small intestine	[8]
Non-ionic surfactant vesicles, deoxycholic acid	Influenza subunit	Oral	The present study demonstrated that oral administration of bilosomes incorporating the influenza sub-unit vaccine could induce a potent antibody response as the parenterally administered vaccine containing the same quantity of antigen	[10]
Sorbitan tristearate, cholesterol, DPPEMCC and CTB	Bovine serum albumin vaccine	Oral	In contrast to FCA, oral administration of bilosomes is convenient and devoid of any adverse effects that are observed with parenteral administration of FCA	[11]
Sorbitan tristearate, cholesterol, dicetyl phosphate, sodium deoxycholate	Hepatitis B Vaccine	Oral	HBsAg loaded bilosomes produced both systemic as well as mucosal antibody responses upon oral administration	[12]
Non-ionic surfactant vesicles, deoxycholic Acid	Influenza A Antigen	Oral	Physically modification (size) of nanocarriers can be used to manipulate immune responses	[13]

DPPE- Dipalmitoyl phosphatidyl ethanoloamine; **SMCC**- Succinimidyl-4-N-maleimidomethyl-cyclohexane-1-carboxylate; **CTB**- Cholera toxin B subunit; **FCA** - Freund's complete adjuvant; **HBsAg**- Hepatitis B surface antigen

between 1 and 10% of healthy adults and 30-90% of infected babies become chronic carriers and on later stages they are at high risk of life-threatening diseases such as cirrhosis and primary hepatocellular carcinoma (HCC). Worldwide, at least one third of the world population have been infected with HBV, over 6% of the world population are chronic carriers and approximately 620,000 people die every year from acute and chronic HBV infection [16]. Globally approximately 4.5 million new HBV infections occur each year of which a quarter progresses to liver disease [17,18].

Immunization is the most successful measure to diminish the global prevalence of hepatitis B. Vaccination is the most important and economically attractive option for healthcare intervention both in terms of cost-effectiveness and benefit-cost ratios. The present concept of vaccination is based on invasive parenteral method which is not completely effective against number of diseases [19]. These traditional needle-based vaccination methods suffer from various drawbacks, like the need for trained personnel to administer vaccines, patient inconvenience and the associated risk of needle-borne infections (AIDS, hepatitis, etc.) due to the use of contaminated needles [20]. The need for trained medical personnel elevated the cost of administration and confined the use of parenteral vaccines in isolated and interior areas. Further, parenterally administered vaccines mainly stimulate a systemic response and antibodies generated in this manner do not always reach to the mucosal surfaces, which is the predominant entry site for most infectious pathogens [21].

These concerns about injectable vaccines are absent in the use of mucosal vaccines. One of needle-free mode of immunization is the oral mucosal immunization that can be commonly and successfully exploited. Features of oral mucosal vaccines i.e. Generation of both mucosal and systemic immunity and better compliance validate this route as effective prophylactic gadget in health care [22]. Oral route is also associated with some major problems including low oral bioavailability of protein related formulations, particularly vaccines. So, to enhance the response and to deliver the vaccine via oral route, suitable delivery vehicle is needed [23].

Several delivery systems which are safe and non toxic and derivatives of commonly used adjuvant have been explored to overcome the above mentioned problems related to delivery of vaccines. They include emulsions, dendrimers, polymeric micro- and nanoparticles, lipid nanoparticles, lipid based vesicles such as liposomes, niosomes, bile salt containing vesicles (bilosomes) and other phospholipids based vesicular constructs and immune-stimulating complexes (ISCOMS). These delivery systems have been shown to protect antigens from degradation in gastrointestinal tract. Particulate systems like nanoparticles of similar size and dimension of the pathogens are efficiently taken up by M cells and hence deliver associated antigen into the antigen presenting cells to provoke strong immune response [24]. Lipid based carriers have also been investigated extensively in the past decades as they resemble with biological membranes and may serve as potential drug and peptide carriers.

The susceptibility of conventional vesicles (both liposomes and niosomes) to bile salt caused dissolution and enzymatic degradation in the gastrointestinal tract remains as the main barrier for their effective oral delivery and led to the development of modified versions of vesicles [25]. Incorporation of bile salts in niosomal formulation could stabilize the membrane against the detrimental effects of bile acids in GI tract [26]. These bile salt stabilized vesicles referred to as "Bilosomes". These allow small quantities of antigen to be effective and both cellular and humoral immune responses can be induced [27]. Further, mannan coating on the novel carrier system can further stabilize the vesicles in gastrointestinal environment as well as can act as a targeting ligand for mannose receptors expressed on macrophages and dendritic cells which are the major antigen presenting cells present in the vicinity of Peyer's patches [28,29].

Characterization of Bilosomes

Bilosomes characterized for their shape and size, a transmission electron microscope (Philips CM-10, Netherlands) used for morphological examination of the bilosomes. A photon correlation spectroscopy used for determination of mean particle size. Particle

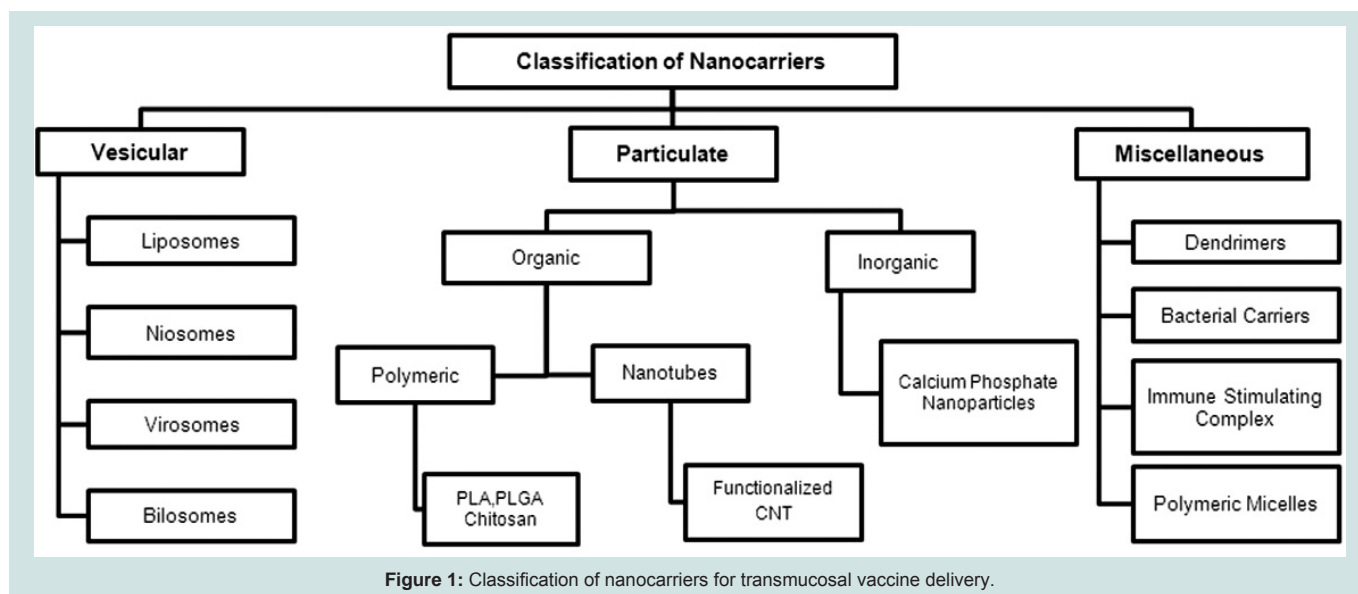


Figure 1: Classification of nanocarriers for transmucosal vaccine delivery.

size analyzer used to carry out vesicle size and size distribution studies [30,31].

Stability Studies of Vesicles in Simulated Gastrointestinal Fluid

Stability determination of coated and uncoated bilosomes can be done in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) in comparison with SGF (pH 1.2) and SIF (pH 7.5) with continuous shaking on wrist action shaker for 2 hour Residual antigen content and number of intact vesicles was then determined [30,31].

Conclusion and Future Perspectives

Vesicular drug delivery systems are emerging with the diverse application in pharmaceuticals, cosmetics and cosmeceuticals and food industries. The delivery of drug directly to the site of infection and it's leading to reduction of drug toxicity with no adverse effects. It also reduces the cost of therapy by imparting better biopharmaceutical properties to the drug, resulting in improved bioavailability and especially in case of poorly soluble drugs. Oral administration remains the favoured method of drug delivery by patients and drug to be successfully absorbed by the body. It requires the active molecule to transit through the stomach and then to be transferred across the intestinal wall to the bloodstream. For a majority of vaccines and biologic therapeutics this is not an option due to a 1% uptake of the dose given. Approximately 1/3 of small molecule drugs also face oral absorption challenges many of these are focussed on CNS targets.

Technology

Scientists at Strathclyde have developed bilosomes and bile salt stabilised vesicles that act as an envelope to protect their contents from the harsh environment of the gut enabling oral administration. The system was well tolerated *in vivo* toxicology studies.

Markets and Applications

Bilosomes has been reported as an effective in the delivery of vaccines (currently under license) and can be applied to the delivery of biological therapeutics and traditional small molecule drugs.

Licensing and Development

The technology is covered by granted patent (US 5,876,721 and EP 0722341B1) currently under license. Patents relating to small molecule and biological therapeutic encapsulation are in preparation [32].

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