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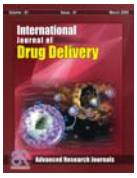

Review

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Chitosan Microspheres as Potential Vaccine Delivery Systems

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Abstract

The recent advances in controlled delivery systems for protein pharmaceuticals such as microspheres, liposomes, pumps and implants, have provided a new avenue for delivery of vaccine antigens. Adjuvants aimed at increasing the immunogenicity of recombinant antigens remain a focus in vaccine development worldwide, there is currently considerable care for the development of chitosan microspheres as controlled release of vaccines, since the major disadvantage of several currently available vaccines is the need for repeated administration. Microspheres prepared from the biodegradable and biocompatible polymers, chitosan have been shown to be effective adjuvants for a number of antigens. This review mainly focuses on chitosan microspheres adjuvant as vaccine delivery systems by summarizing our and other research groups' investigation on properties of microspheres formulation encapsulating several kinds of antigens. The results indicate that compared with commonly used PLA and PLGA, chitosan biomaterial has several potentials in vaccine delivery systems. Chitosan microspheres can control the rate of release of entrapped antigens and therefore, offer generation adjuvant to replace or complement existing aluminium salts for vaccine potential. The review mainly aims to promote the investigation of chitosan microspheres adjuvant for antigens for world wide researcher.

Keywords: Tetanus toxoid; Chitosan microspheres; Vaccine delivery system; Biodegradable polymers.

Introduction

Immunization has arguably been the most important way of protection against a number of devastating viral and bacterial infections [1]. Vaccine research is often focused on the identification and application of novel antigens. The response to those antigens is routinely optimized by assessing a variety of delivery methods, including variation of the adjuvant used, the dose and number of infections and the route of delivery. The application of new delivery systems to vaccination may allow effective utilization of vaccine antigens that have previously not been able to induce adequate or appropriate responses as well as improving the responses to existing vaccines [2].

Aluminium phosphate and aluminium hydroxide, which are currently the approved adjuvant for human vaccination in US, are widely used

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vaccine adjuvants for humans at present. However, the use of alum-type adjuvant for immunization has some disadvantages [3-7]. Although alum is efficient at increasing humoral immunity, cell mediated immunity appears to be only slightly affected. Moreover some viral antigens are poor immunogens in alum [4, 8]. Also, alum is an undesirable adjuvant since it stimulates local production of granulomas and induces inflammation [7]. Conventional alum-type vaccines requires multiple recall injections (e.g. Diphtheria, pertussis, tetanus, hydrophobia and hepatitis B) at appropriately timed intervals in order to obtain long lasting and optimal immune response. Therefore, development of more efficient and safe adjuvant / vaccine delivery systems requiring single administration to obtain high and long lasting immune responses is of primary importance. A new generation of more effective adjuvants [9], including liposomes, muramyl peptide and ISCOMs (Immunostimulatory Compounds), have been proposed to replace alum.

In recent years there have been various attempts to demonstrate new immunization strategies to induce higher level and larger duration of immune responses following parenteral and / or oral administration. One of the means used to improve immunologic response has been to provide prolonged antigen release [5]. Controlled drug delivery technology using biodegradable polymers as carriers represents on of the most rapidly advancing areas of science. Controlled delivery systems consisting of biodegradable microspheres can potentially delivery either the antigens or adjuvants to the desired location at predetermined rates and durations to generate an optimal immune response. The carrier may also protect the vaccine from degradation until it is released. Other potential advantages of the controlled delivery approach include reduced systemic side effects and the possibility of coencapsulating multiple antigenic epitopes or both antigen and adjuvant in a single carrier. Biodegradable polymers provide sustained release of the encapsulated antigen and degrade in the body to nontoxic, low-molecular-weight products that are easily eliminated [5, 10]. The microspheres vaccine delivery system based on biodegradable polylactide (PLA) and polylactide-co-glycolide (PLGA) has been extensively investigated due to the many advantages of the controlled-release delivery system [3, 5, 10-17].

The PLA and PLGA are biodegradable and biocompatible polymers, which are nonimmunogenic and have a long history of safe use in humans as sutures and as controlled delivery systems [11, 18-20]. The choice of PLA and PLGA as the matrix for vaccine formulations is based on its long-term safety in humans, its biodegradability, and the commercial availability of a variety of polymers of different molecular weights and monomer ratios [21]. Nevertheless, PLA and PLGA have some drawbacks, resulting from their hydrophobic nature. The difference in physico-chemical properties between hydrophilic antigens and hydrophobic polymer matrix leads to a lower encapsulation efficiency within microspheres, and a higher burst effect of antigen release from microspheres. Furthermore, during the initial vaccine release phase in vivo, the hydrophobic PLA or PLGA prevents the penetration of water into the center of the microspheres, thus forming an acidic microenvironment due to the accumulated acidic breakdown products, such as lactic and glycolic acid end groups.

Chitosan derived by the deacetylation of chitin, which is a polymer of D-glucosamine and N-acetyl D-glucosamine. Chitosan is well known for its hydrophilic, biocompatible, biodegradable and non-toxic properties [22, 23]. Chitosan particle delivery system can reduce the clearance rate from the nasal cavity, there by increasing the contact time of the delivery system with the nasal mucosa [24]. Chitosan suspensions or micro and nanoparticles have been reported to have immune stimulating activity such as increasing accumulation and activation of macrophage and poly morphonuclear cell, promoting resistance to infections of microorganisms, and inducing cytokines [25].

Although, the currently available vaccines represent an outstanding success story in modern

medicine and have had a dramatic effect on morbidity and mortality worldwide, it is clear that improvements are required in the current vaccine delivery technologies to control the conventional approaches [26].

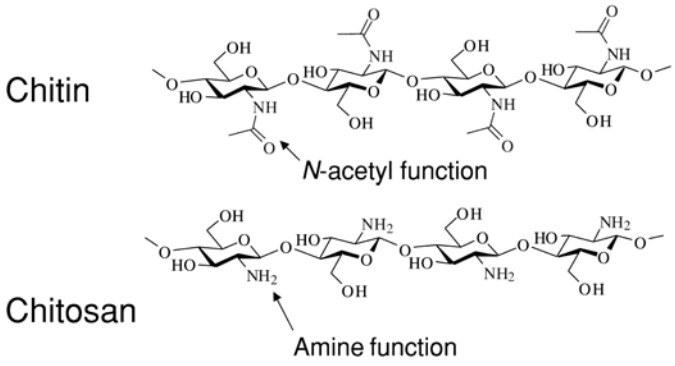

Figure 1. Structure of chitin and chitosan

Emergence for New and Improved Vaccines

Despite the success of vaccines, there is a clear need for the development of new vaccines against infectious diseases for which none are yet available, or are inadequate, including HIV, HCV, Neisseria Meningitidis type 3, Tuberculosis and Malaria. Unfortunately, these pathogens have proven exceptionally difficult to control using traditional approaches to vaccine development and novel approaches will be needed. Vaccines are also needed to protect against emerging or reemerging infectious diseases, including West Nile, SARS, Ebola, Hanta and Dengue viruses. In addition, improved vaccines are needed to protect against the thread of pandemic strains of influenza virus and the continued growth and speed of antimicrobial resistant organisms. In addition, vaccines may be required to protect against the threat of bioterrorism [27]. Moreover, there is an increasing awareness that infectious agents can cause chronic diseases, which might be prevented or treated with novel vaccines.

Hence vaccines may also be considered as potential therapeutic agents to treat established infections. It is clear that novel vaccine delivery technologies will be required to enable the development of these new vaccines, particularly those designed as therapies against chronic infections or cancers. The safety profiles of therapeutic vaccines may look very difficult from traditional vaccines, which are used to protect against infection. Therapeutic vaccines, particularly if used in an oncology setting, or to treat established life-threatening infectious diseases, would most likely be able to sustain a higher level of adverse effects without damaging the marketability of the product.

Examples of Alternate Approaches to Vaccine Delivery

1) Antigen delivery system / Adjuvants: Alum, MF59, PLG, Liposomes, Virosomes, etc.
2) Adjuvant delivery system: PLG, MF59, ISCOMS, etc.
3) DNA vaccine delivery systems: PLG, Gene gun, Alpha viruses, etc.
4) Nonreplicating viral vectors: Alpha viruses, etc.
5) Live bacterial and viral vectors: Salmonella, Adenoviruses, etc.
6) Intranasal vaccines: LT mutants and / or bioadhesive polymers etc.
7) Oral vaccines: LT mutants, Enteric coated formulations, etc.
8) Topical vaccines: Vaccine patches (Lomai)
9) Micro needles: Onvax (Becton Dickinson), Macro flux (Alza), etc.
10) Needle free devices: Epidermal powder immunization (Powderject), Bioreactor etc.

Although the term vaccine "adjuvants" and "delivery systems" have often been used interchangeably, a clear distinction can often be made and the respective roles of each can be more clearly defined. Classically, adjuvants have been defined by what they do, which is to enhance the immune responses to antigens, rather than by how they achieve this.

Chitosan Microspheres

Characteristics of Chitosan

Chitosan is a natural polysaccharide consisting of glucosamine and N-acetyl glucosamine. It can be

derived through the partial deacetylation of chitin, the major compound of exoskeletons in crustaceans. The term chitosan refers to a series of polymers with different molecular weights, viscosity, degrees of deacetylation, pKa, etc. The molecular unit of chitosan has one amino group and two hydroxyl groups that are potentially capable of reacting with an acidic medium. The amino group in chitosan has a pKa value of ~6.5; hence, chitosan is positively charged and is soluble in an acidic solution with a charge density that is dependent on the pH and the degree of deacetylation. The presence of an amino group in chitosan enables it to chemically react with anionic systems, thereby resulting in the modification of the physicochemical characteristics of such combinations [22]. In addition, the free amino group in chitosan is readily available and reacts with a number of negatively charged polymers. Chitosan is one of the most promising polymers because of its nontoxic, polycationic, biocompatible, and biodegradable nature, and particularly due to its mucoadhesive and permeation-enhancing properties.

The strong mucoadhesive property of chitosan is most important for drug delivery through the mucosal routes. In addition, the interaction of the positively charged chitosan with the negatively charged mucin layer and the tight junctions facilitates the paracellular transport of hydrophilic macromolecules by opening the tight junctions of the mucosal barriers[28-31]. The strong mucoadhesive properties of chitosan point to its potential as a permeation enhancer for mucosal drug delivery. Nasal and oral drug delivery researches have confirmed that significantly higher amounts of macromolecules can be absorbed across the mucosal barrier after co-administration with chitosan [28, 32]. The absorption-enhancing effect of chitosan has been found to be due to the combination of improved mucoadhesion between the formulation and the nasal tissues and the transient effect of chitosan on the paracellular pathways. Dodane et al. demonstrated that chitosan has an effect in modifying paracellular transport [30]. There are several researches that show the efficacy of chitosan as an adjuvant and delivery system for mucosal vaccines. The *Bordetella pertusis* filamentous haemagglutinin and recombinant pertusis toxins have been shown to induce strong antigen-specific systemic and mucosal immune responses after IN administration with chitosan [33]. Diphtheria toxin nasally co-administrated with chitosan induced systemic and local immune responses [34]. In several researches, mucosal vaccination with chitosan induced cell-mediated immune responses as well as humoral immune responses.

In a study conducted by Xie et al., the H. pylori vaccine with chitosan as an adjuvant via oral delivery induced both helper T cell 1 (Th1)- and helper T cell 2(Th2)-type immune responses [35]. The intranasally delivered chitosan-diphtheria vaccine in primed animals was found to induce a mixed Th1/Th2 response,which indicates the induction of both humoral and cellular immune responses [22]. Chitosan was also shown to have immune-stimulating activity, such as increasing the accumulation and activation of macrophages, promoting the production of cytokines, and enhancing cytotoxic T lymphocyte (CTL) responses [36]. The mucociliary clearance rate can be decreased by the use of mucoadhesive polymers. Several studies showed that chitosan prolonged the residence time of nasally delivered drugs at the absorption site [37].

Mucoadhesive microspheres

Particulate carrier technology offers a valuable advance for use as vaccine delivery systems by the introduction of vaccines to the carriers such as nano/microspheres, emulsions, liposomes, virosomes, immune stimulating complex (ISCOM), virus like particles (VLPs), etc. Microspheres which constitute a significant part of these particulate vaccine delivery systems have several useful attributes to stimulate host immune system. First, the most useful property of microspheres as vaccine delivery systems is to offer an optimum size for particle trafficking into the body as well as uptake by antigen presenting cells (APCs). It has been well documented that

the drug carrying microspheres with an appropriate size can be internalized by APCs thereby allowing for easy transport of the vaccine formulations across the cell membrane [38]. The sizes of microparticles were an important parameter influencing the efficacy to enhance immunogenicity, because small particles (<10 μm) appeared to be more immunogenic than large ones (>10 μm) [39, 40]. A second valuable property of microspheres is to increase the residence time of vaccines at the mucosal surface compared to solutions. It has been reported that microspheres exert a direct effect on the nasal mucosa because the epithelial cells dehydrate causing the tight junction to separate by absorbing water from mucus and swelling [41]. The increased residence time of microspheres at the mucosal surface may facilitate the increased uptake of vaccine formulation incorporated with the microspheres by more increased contact time between the vaccine and the mucosal membrane.

The microspheres with a mucoadhesive property can offer additional advantages that may help to prolong residence time and improve uptake of vaccines incorporated with them. Chowdary et al. well documented the advantages of mucoadhesive drug delivery systems such as bioavailability improvement of drugs, absorption enhancement of macromolecules and prolonged residence time at the site of application [42]. Chitosan among these mucoadhesive agents is the most widely used natural polymer for potential application of mucosal vaccine delivery. The chitosan microspheres as a delivery system for oral vaccination were evaluated in several studies. In a study conducted by Tian et al., fish immunized by oral vaccination with a plasmid DNA (pDNA) containing major capsid protein (MCP) gene of lymphocystis disease virus (LCDV) encapsulated in chitosan microspheres showed significantly enhanced systemic immune responses in comparison with fish vaccinated with naked pDNA [43]. The chitosan encapsulated tetanus toxoid (CS-TT) microparticles have been shown to induce antigen specific IgA in intestinal lavage, faeces, intestinal washings and strong antigen specific IgG in the systemic circulation after oral immunization [44]. Since chitosan polymers have already been used for a variety of biomedical purposes, including the preparation of controlled drug delivery systems, including proteins [45], it was an excellent choice for a vaccine delivery system. Microspheres represent an attractive approach to vaccine delivery since it has been shown on many occasions that microspheres are taken up efficiently by Antigen Presenting Cells (APC) in-vitro (39) and in-vivo [40]. In addition, microspheres have also been shown to be taken up by APC, which then migrated to the T-cell area of local lymph nodes and differentiated in to Dentritic cells (DC) [39].

Compared with PLA and PLGA, Chitosan is a new cheap biomaterial and fewer reports are published on its use as a drug delivery carrier. In last 5 years, our research groups have focused on studying the preparation of chitosan microspheres as a suitable vaccine delivery system [46].

Conclusion

Biodegradable chitosan microspheres are extremely flexible delivery systems capable of encapsulating a wide range of antigens. A single injection or oral administration of chitosan microspheres could provide a sustained, higher or equal antibody titer than an alum adsorbed vaccine. Furthermore, the chitosan polymer improves cellular immunity, a property which alum lacks. Therefore, chitosan polymer has potential use as a new adjuvant instead of alum, and, the microsphere formulation is very suitable to be as vaccine delivery system. Chitosan is a novel biomaterial, and it is very worth studying.

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