



The use of soluble polymers and polymer microparticles to provide improved vaccine responses after parenteral and mucosal delivery

Stanley S. Davis*

Institute of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK

Abstract

It is important when developing new vaccine systems to give proper attention to the question of delivery. In some cases the judicious choice of a delivery system can provide a greatly enhanced immune response and avoid the need to use a vaccine adjuvant. Delivery systems that have been developed originally for the administration of challenging drug can be used with success for vaccines. Polymer microspheres and lamellar particle based on the biodegradable materials polylactide and polylactide co-glycolide can be employed for the improved parenteral and mucosal administration of antigens. Likewise soluble biopolymers such as chitosan can be used for the improved nasal delivery of various antigens as well as DNA. Results from animal studies and recent clinical trials are provided.

© 2005 Published by Elsevier Ltd.

Keywords: Chitosan; Microspheres; Nasal

1. Vaccine delivery

A variety of materials are now available for use as ‘antigens’ in the development of novel vaccine systems. These include whole organisms (both live and inactive), proteins, lipopolysaccharides and DNA. In addition, many new immunological adjuvants have been described, which may well have advantages over conventional materials such as alum. However, an essential part of the development of a successful vaccine product can also include the choice of an appropriate delivery system. Indeed in some cases the judicious combination of antigen and delivery system may induce a strong immune response without the need for an adjuvant. In our work on vaccine delivery, we have employed systems that were originally developed for the delivery of drugs. The choice of the delivery system has been based on a thorough understanding of the disease, the nature of the antigen (physicochemical properties, stability, etc.) the response required and the preferred route of administration. Both parenteral and mucosal vaccine systems have been explored and candidate systems have been evaluated in animal models and in man. Polymeric

materials that have been approved for use in man have been of special interest.

2. Biodegradable synthetic polymers

There has long been interest in the use of biodegradable polymers for the controlled delivery of drugs parenterally. Microspheres and implants based on polylactic acid-co-glycolide have been investigated in detail and products containing peptides such as luteinising releasing hormone, and growth hormone have been introduced successfully to the market [1]. We were one of the first groups to use polymeric microspheres for the parenteral and oral administration of vaccines [2]. Good data have been obtained in small animal models but results obtained in large animal models and in man have been less than satisfactory, especially for oral administration. It is appreciated that polymer microspheres have certain limitations. Few polymers are approved by the regulatory authorities (for example polylactide, polylactide co-glycolide). The loading of antigen into the microspheres can be very low (less than 1% w/w) and the material so loaded can be degraded during processing by strong shear forces or after loading when the polymer breaks down and releases

* Tel.: +44 115 9515121; fax: +44 115 9515122.

E-mail address: stanley.davis@nottingham.ac.uk.

acid components. We have been able to develop systems with higher loading and improved stability through the incorporation of a second water-soluble polymer such as polyethylene glycol [3]. Various antigens have been loaded successfully with high rates of incorporation. Slow controlled release of the antigen has been observed leading to a good immune response after parenteral administration. It is believed that the water-soluble polymer is released together with the antigen and that the water insoluble polymer acts as a sponge-like matrix. Recently Yeh and Chiang [4] have described the loading of *Vibrio cholerae* into this type of system to provide a whole cell vaccine. Mice were dosed orally and then subjected to a lethal i.p. challenge. The novel microsphere systems outperformed conventional microspheres.

For any microsphere system a certain proportion of the 'loaded' antigen will be found on the surface of the particles rather than entrapped in the core. This surface material is normally released quite quickly to provide an initial dose followed by the slower release of the material from the core. We have found that some antigens are very difficult to load into particles. Studies with whole influenza virus showed that there was almost nothing in the core; the virus instead was firmly attached to the particle surface. Nevertheless, a good immune response was obtained. We reasoned, therefore that for some antigens, it might be more appropriate and certainly simpler and cheaper to exploit surface adsorption rather than attempt to load the material into particles. In order to provide a high surface for adsorption we developed polymeric lamellar particles by the controlled precipitation of a crystalline polymer such as polylactic acid (an approved material). It is a simple versatile process that is easily scaled-up. The antigen is adsorbed to the particle under mild conditions and there is no exposure of the antigen to organic solvents or shear. Good immune responses have been obtained via both systemic and mucosal routes in the mouse model. For example, enhanced and durable immunity was obtained with influenza virus administered i.m. and the responses were the same or better than with alum. Immunity across strains was demonstrated (Harbin, Sichuan, N/chang) [5]. The lamellar system has been optimized in terms of 'formulation factors' such as particle size, polymer properties (molecular weight) and surface characteristics. The last factor has been of special interest. Antigen adsorption and release has been controlled by modification of the surface by making it more hydrophilic or hydrophobic. Likewise, it has been possible to make the surface positively charged (instead of the usual negative charge) and in this way to adsorb DNA for the delivery of gene based vaccines.

Interesting results have been reported by Venkataprasad et al. [6] who adsorbed the 38 kDa protein antigen (50 mg) from *Mycobacterium tuberculosis* to lamellar particles and then administered them to mice s.c. A strong Th1 response and high levels of gamma interferon were observed. A synthetic peptide was also investigated.

We have also conducted initial experiments on the intranasal and oral administration of lamellar particles carrying

adsorbed influenza virus (trivalent). Good specific IgA and IgG levels were obtained in a mouse model.

Clearly, it will be of interest to test these polymers based systems in man following parenteral and mucosal administration, notwithstanding the difficulties of undertaking clinical investigation with new products. Before such investigations are conducted it will be important to have a better understanding of the delivery issues and in particular the preferred site of uptake for optimal effect. For instance, much is known about the nose associated lymphoid tissue in the rodent but what is the equivalent in man. Where a nasal spray should be directed? To Waldeyer's ring perhaps. Similar considerations apply to the oral administration of vaccines to man. The gut associated lymphoid tissue has been well investigated in rodent and rabbit models but what is the situation in larger animals and particularly man? Oral vaccines have usually performed poorly in clinical studies but this is perhaps no wonder. Has the vaccine been delivered properly to the optimal site and has the antigen been protected and then released at the appropriate site and at the appropriate time? It can be argued that the terminal ileum is the best place in man for vaccine delivery because of the preponderance of Peyer's patches but there is more lymphoid tissue in the human colon. As yet there has not seemed to be a proper systematic study conducted to ascertain the advantages of delivering oral vaccines to the specific regions of the human gastrointestinal tract. This can be readily achieved and indeed is now standard practice when assessing the absorption characteristics of new drug candidates. Advanced capsule systems can be used to release a payload at designated sites, together with materials that may enhance vaccine stability and/or uptake [7].

It can be argued that improved (targeted?) delivery of mucosal vaccines may well provide an effective answer to poor responses and could avoid the need for the addition of adjuvants that may be reactogenic. The recent problems with nasal influenza virus vaccines are a case in point. [8]. As will be discussed below the performance of nasal vaccine can be improved by the use of bioadhesive materials that have a role in slowing mucociliary clearance and thereby holding the delivery system at a preferred site for an extended period of time.

3. Mucosal vaccines and biopolymers

The administration of vaccines to mucosal surfaces would confer considerable advantages since mucosal surfaces are the sites through which most antigens are encountered. In our studies we have paid special attention to the nasal route and once again we have applied technologies that were developed originally for the improved delivery of challenging drugs (e.g. insulin, calcitonin etc.). Mucosal vaccines have been developed for a range of different antigens (to include DNA) using the biopolymer chitosan. These systems have been tested in animal models and some more recently in man.

Chitosan is a cationic polymer that it derived from the natural material chitin by a process of partial deacetylation. Chitosan has some interesting properties when used for drug delivery. It is bioadhesive and thereby slows down the clearance of formulations from the nasal cavity. Of equal importance is the ability of the material to open up the tight junctions between cells and allow improved access of drugs (and antigens) to the underlying tissues (for the case of antigens perhaps to improve contact of antigen with intraepithelial and submucosal lymphocytes)

This effect of chitosan on tight junctions is transient and, as far as can be ascertained, chitosan is non-toxic and non-immunogenic. The material is used in pharmaceutical formulations and is available at high quality (GMP standard) [9].

Initial studies have been performed in a mouse model using the purified surface antigen (PSA) from B/Panama influenza. Good immune responses were obtained as demonstrated by serum IgG anti-HA levels, and nasal wash secretory IgA anti-HA levels. Antibody secreting cells were also determined. A human clinical trial has now been conducted in 60 subjects using trivalent influenza vaccine: A/Sydney/5/97, A/Beijin/262/95, B/Yamanashi/166/98. Two nasal formulations containing chitosan were compared to a conventional intramuscular (i.m.) product. A four-fold increase in HI value relative to pre-dose HI value indicated an effective immune response to the nasal vaccine. The responses were slightly lower or similar for intranasal as compared to i.m. administration. Observed HI values of 40 or larger following nasal administration indicated protective level to the antigen and the number of volunteers with protective levels was similar for i.m. and intranasal dosing. This new nasal influenza vaccine was well tolerated by the volunteers [10].

Further studies with chitosan have been conducted in mice, guinea pigs, sheep and man with a formaldehyde stabilised recombinant diphtheria toxin (CRM 197). Naive and primed subjects have been used together with challenge tests. The chitosan nasal formulations have been in the form of solutions and powders. Various read-outs have been obtained such as serum antibodies, lung lavage and nasal wash antibodies, neutralizing antibodies (Vero cell assay), T cell proliferation tests and the measurement of relevant cytokines. Key formulation factors such as the ratio of antigen to chitosan and the possible advantage of powder formulations were also assessed as was the importance of administering the antigen and the chitosan at the same time. In a 'pulse-chase study' chitosan was administered one day earlier than CRM 197. A significant decrease in serum IgG response was found as compared to simultaneous administration. Chitosan given alone intranasally gave no serum IgG response. A chitosan powder formulation containing CRM 197 has been evaluated recently in a human clinical trial to evaluate safety, tolerability and immunogenicity. Two nasal doses were given 28 days apart to subjects previously immunized (>5 years) with a conventional diphtheria vaccine.

A single nasal immunization was well tolerated and boosted antitoxin neutralizing activity which could be further boosted by a second immunization. The neutralizing activity far exceeded accepted protective levels and was equivalent to that induced by standard i.m. vaccines and was significantly greater than intranasal immunization with CRM 197 in the absence of chitosan. Interestingly, unilateral intranasal immunization induced circulating antitoxin antibody-secreting cells, but a nasal antitoxin sIgA response was seen only after the second immunization and only in the vaccinated nostril [11].

Chitosan can also be used to deliver DNA. It is a cationic material and it will therefore interact with negatively charged DNA to form insoluble complexes. Under appropriate conditions these complexes are in the form of sub-micron particles–nanoparticles. The ratio of DNA to chitosan can be altered so as to control the particle size of the particles and more importantly the surface charge. It has been found that particles of a size around 100 nm and a charge of greater than +20 mV can provide good transfection of cells. This corresponds to a DNA: Chitosan ratio of 1:5. A nasal DNA influenza vaccine based on chitosan has been evaluated in a challenge study in primed mice. DNA expressing influenza haemagglutinin (HA) and nucleoprotein (NP) of A/Sichuan/2/87 (H3N2) virus was used. A priming dose of 100 µg naked DNA was given i.m. followed by a boosting dose of 20 µg of various formulations either intranasally or i.m. Intranasal challenge with 50 times the 50% infectious dose of the virus was performed 2 weeks after boosting following a nasal wash and sampling of serum. Good antibody levels were seen and in the challenge study a dramatic reduction in virus shedding was seen for all animals given the DNA vaccine, whether by nasal or i.m. routes. This study was followed up by a more detailed investigation on the nasal delivery of chitosan–DNA plasmid complexes expressing CTL epitopes from the M2 protein of respiratory syncytial virus (RSV). Protective CTL responses were induced in Balb/c mice after nasal administration [12]. The DNA complexes were given on three separate occasions and then 2 weeks after the last immunization the mice were challenged nasally with 10⁵ pfu of RSV. Four days after the challenge, RSV was harvested from lungs and assayed on HEp-2 cells. A dramatic reduction in virus titre was seen for the nasal vaccine. This effect was similar to that for the positive controls in the form of intradermal administration of the DNA complex and the s.c. administration of the peptide.

References

- [1] Okada H. One- and three-month release injectable microspheres of the LH-RH superagonist leuprorelin acetate. *Adv Drug Deliv Rev* 1997;28(1):43–70.
- [2] O'Hagan DT, Jeffery H, Roberts MJ, McGee JP, Davis SS. Controlled release microparticles for vaccine development. *Vaccine* 1991;9(10):768–71.

- 278 [3] Yeh MK, Davis SS, Coombes AG. Improving protein delivery from
279 microparticles using blends of poly(DL lactide co-glycolide) and
280 poly(ethylene oxide)-poly(propylene oxide)copolymers. *Pharm Res*
281 1996;13(11):1693–8.
- 282 [4] Yeh M, Chiang C. Inactive *Vibrio cholerae* whole-cell vaccine-loaded
283 biodegradable microparticles: in vitro release and oral vaccination. *J*
284 *Microencapsul* 2004;21(1):91–106.
- 285 [5] Jabbal-Gill I, Lin W, Jenkins P, Watts P, Jimenez M, Illum L, et al.
286 Potential of polymeric lamellar substrate particles (PLSP) as adju-
287 vants for vaccines. *Vaccine* 1999;18(3–4):238–50.
- 288 [6] Venkataprasad N, Coombes AG, Singh M, Rohde M, Wilkinson K,
289 Hudecz F, et al. Induction of cellular immunity to a mycobacterial
290 antigen adsorbed on lamellar particles of lactide polymers. *Vaccine*
291 1999;17(15–16):1814–9.
- 292 [7] Wilding II, Hirst P, Connor A. Development of a new engineering-
293 based capsule for human drug absorption studies. *Pharm Sci Technol*
Today 2000;3(11):385–92.
- [8] Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, et al. 294
Use of the inactivated intranasal influenza vaccine and the risk of 295
Bell's palsy in Switzerland. *N Engl J Med* 2004;350(9):896–903. 296
- [9] Illum L. Chitosan and its use as a pharmaceutical excipient. *Pharm* 297
Res 1998;15(9):1326–31. 298
- [10] Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan 299
as a novel nasal delivery system for vaccines. *Adv Drug Deliv Rev* 300
2001;51(13):81–96. 301
- [11] Mills KH, Cosgrove C, McNeela EA, Sexton A, Giemza R, Jabbal- 302
Gill I, et al. Protective levels of diphtheria-neutralizing antibody in- 303
duced in healthy volunteers by unilateral priming-boosting intranasal 304
immunization associated with restricted ipsilateral mucosal secretory 305
immunoglobulin A. *Infect Immun* 2003;71(2):726–32. 306
- [12] Iqbal M, Lin W, Jabbal-Gill I, Davis SS, Steward MW, Illum L. 307
Nasal delivery of chitosan-DNA plasmid expressing epitopes of res- 308
piratory syncytial virus (RSV) induces protective CTL responses in 309
BALB/c mice. *Vaccine* 2003;21(13–14):1478–85. 310

UNCORRECTED PROOF