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Note

Effect of Vitamin E TPGS on immune response to nasally delivered diphtheria toxoid loaded poly(caprolactone) microparticles

S. Somavarapu, S. Pandit, G. Gradassi, M. Bandera, E. Ravichandran, Oya H. Alpar*

Vaccine Delivery Group, Centre for Drug Delivery Research, University of London, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK

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Abstract

The nasal mucosa has many advantages as a potential site for drug and vaccine delivery. The present study has sought to exploit this route of delivery using microparticles composed of D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) as a matrix material blended with poly(caprolactone) for nasal immunisation with diphtheria toxoid. Particles were prepared by a double emulsion method, followed by spray drying and the effect of TPGS on size, zeta potential, loading and release of antigen was assessed. Particles composed of TPGS–PCL blends were spherical, smooth and monodisperse, displaying increasing yields after spray drying with increasing concentrations of TPGS. The immune response to diphtheria toxoid loaded PCL-TPGS microspheres after nasal administration was shown to be higher than that achieved using PCL microspheres alone. We conclude that TPGS shows significant potential as a novel adjuvant either alone or in combination with an appropriate delivery system.

Keywords: Microspheres; Vitamin E TPGS; PCL; Emulsification; Spray drying; Diphtheria toxoid; Intra nasal

The potential of the nasal route as a mucosal site for drug and vaccine delivery has now been firmly established. The nasal mucosa possesses many advantages for drug and vaccine delivery, including a highly vascularised epithelium of considerable surface area. In addition, conditions in the nasal cavity are less harsh than those present in the gastrointestinal tract because there is lower enzymatic activity and reduced exposure to extremes of pH. The existence of nasal-associated lymphoid tissue (NALT), which has a role that is analogous to that of the gut associated lymphoid tissue (GALT), is thought to be important for the uptake of particulate carriers for the purposes of immunisation. Several studies have demonstrated the potential

* Corresponding author. Tel.: +44 207 753 5928; fax: +44 207 753 5942.
E-mail address: oya.alpar@ulsop.ac.uk (O.H. Alpar).

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of poly(lactic-co-glycolic acid) [PLGA] microparticles as vaccine carriers for intranasal (IN) delivery. The IN immunisation of microencapsulated *Bordetella pertussis* antigens has been shown to give protective immunity in mice (Shahin et al., 1995). Immunisation with microparticles IN has also induced protection in mice against aerosol challenge with ricin toxin (Yan et al., 1996). In our laboratory, the IN delivery of tetanus toxoid in microparticles has been shown to induce markedly higher systemic and local immune responses than the free toxoid (Almeida et al., 1993; Eyles et al., 1999).

In this work D-α-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS, referred to as TPGS herein) has been used as a matrix material, blended with poly(caprolactone) [PCL] polymer in the preparation of microparticles entrapping diphtheria toxoid. TPGS is a water soluble derivative of Vitamin E, and has an amphiphilic structure; the tocopheryl part of the molecule is lipophilic and the PEG portion is hydrophilic. Thus, despite its bulky shape and large surface area TPGS acts as a surface active agent, being miscible in both water and oil (Mu and Feng, 2003). In a study conducted by Mu and Feng (2003), it was found that hydrophobic polymers, such as PLA and PLGA, could be blended with TPGS thus forming a matrix material to improve the controlled release of drug from the particles. The aim of the present work was to examine the effect of varying the TPGS content on particle size, zeta potential, diphtheria toxoid loading and release, as well as the immune response to nasally delivered microparticles.

Diphtheria toxoid loaded microspheres were prepared by emulsification followed by spray drying as follows. The aqueous component of the primary emulsion consisted of 260 μl diphtheria toxoid (19.3 mg/ml, Pasteur Merieux, France) and 750 μl of 7.5% (w/v) poly(vinyl alcohol) [PVA, 13–23 kDa, Sigma, UK], thus resulting in 5.018 mg antigen per formulation; the organic polymer component contained 250 mg poly(caprolactone) [PCL, 42.5 kDa, Sigma, UK] and varying concentration of TPGS (a kind gift from Eastman, USA), both dissolved in 5 ml dichloromethane (BDH, UK). The primary emulsion was formed by mixing with an Ultraturrax homogeniser for 2 min at 24,000 rpm before being added to 30 ml of 2.5% (w/v) PVA to produce a w/o/w double emulsion (Silverson homogeniser, 5 min at 10,000 rpm). This secondary emulsion was then spray-dried with a Mini Büchi B-191 laboratory spray-dryer (Büchi Laboratorium AG, Switzerland) using a 0.5 mm nozzle, and the following process parameters: inlet temperature, $40 \pm 2\,^{\circ}C$; outlet temperature, $26 \pm 2\,^{\circ}C$; aspirator setting, 50%; pump setting, 5 ml/min and spray flow, 600 l/min. The size of the microparticles was determined by using a Malvern MasterSizer/E (Malvern Instruments, UK). The zeta potential of the particles was measured using a ZetaSizer (Malvern Instruments, UK). Scanning electron microscopy (SEM) was used to analyse the size, morphology and surface properties of microspheres. The BCA assay was used to determine the loading efficiency and in vitro release of the microspheres after 5 mg of particles were digested with 500 μl DCM for 30 min and then the diphtheria toxoid was extracted into deionised water. For the in vitro release study, 5 mg of particles were suspended in PBS release medium and maintained at $37\,^{\circ}C$ in an incubator for the duration of the experiment. The amount of protein released is presented as a percentage of the original mass loaded. The in vivo immunisation study used four groups of female BALB/c mice (∼25 g, 6 weeks old) to assess the ability of PCL particles alone, PCL with 10% TPGS, PCL with 20% TPGS and free diphtheria toxoid to elicit an IgG serum specific antibody response. Mice were anaesthetized prior to receiving a 20 μl intranasal dose (divided equally between the nostrils) via micropipette. For both diphtheria toxoid loaded particles and the antigen alone an initial dose of 10 μg diphtheria toxoid was given on day 1, followed by a 5 μg boost on day 28. Blood samples from the tail vein were taken on days 15 and 45 and the serum specific antibody response assessed by enzyme-linked immunosorbent assay (ELISA).

The mean diameter of the microspheres was found to be between 2 and 3 μm with 90% of particles in the range of 2–3 μm (Table 1). Fig. 1 shows SEM images of microspheres in sample A1 which revealed spherical particles with varying smoothness of surface. When the concentration of TPGS in the organic phase was increased to 20 and 40% (w/v) formulations A3 and A4 respectively, the surface morphology of the particles was found to be more smooth and less 'pitted' as compared to formulation A1.

Generally the yield obtained from spray drying is low. In our work a 32% yield was obtained for PCL only microspheres. When TPGS–PCL blends were used the

Table 1
Summary of physicochemical characteristics for PCL and TPGS–PCL blend microspheres loaded with diphtheria toxoid

Code	TPGS (w/w)	Yield (%)	Mean volume diameter (μm) ± S.D.	Zeta potential (mV) ± S.D.	% loading efficiency ± S.D.	% burst release after 1 h ± S.D.
A1	–	32	2.33 ± 1.29	−2.30 ± 0.20	61.38 ± 0.85	43.30 ± 0.91
A2	10%	39	2.11 ± 1.10	−5.40 ± 0.10	59.1 ± 0.51	41.97 ± 0.71
A3	20%	42	2.43 ± 1.36	−6.40 ± 0.20	50.93 ± 0.57	36.57 ± 0.47
A4	40%	45	2.59 ± 1.65	−6.80 ± 0.30	61.03 ± 0.86	29.64 ± 0.28

yield gradually increased with increasing concentrations of TPGS. For 10, 20 and 40% (w/v) TPGS the yield was 39, 42 and 45% respectively. This contradicts the results of Mu and Feng, where the yield was decreased with polymer–TPGS blends. This may be attributed to different processing parameters such as preparation method (the authors used solvent evaporation method), polymer used (poly(lactic acid) [PLA] and PLGA versus PCL) and the emulsifying agent.

The loading efficiency of the formulations made with PVA as the emulsifier (A1–A4) was between 50 and 60%; the 20% (w/v) TPGS–PCL blend had the lowest loading efficiency of 50% although the differences were not significant. These results show that the TPGS–PCL blend made no significant difference in the loading efficiency of diphtheria toxoid. The formulation made with PCL alone (A1) showed the highest burst release (∼44%), whereas formulations made with TPGS–PCL blends showed reduced burst release. The burst release decreased significantly with increasing TPGS concentration. This could be due to the difference in DT distribution in PCL only and PCL-TPGS microspheres.

All the PCL microsphere formulations resulted in better immune responses compared to free DT (Fig. 2). At day 45, the diphtheria toxoid specific serum antibody responses for PCL-TPGS blend microspheres was 13-fold higher than that achieved using the diphtheria toxoid loaded PCL microspheres alone. We speculate that the differences between these formulations may be even more marked after a longer period of time.

TPGS has previously been shown to function as a safe mucosal adjuvant (Alpar et al., 2001), but its mechanism of action is not well understood. The antioxidant properties of TPGS may in part be responsible for its observed adjuvant effect, as it is known to facilitate

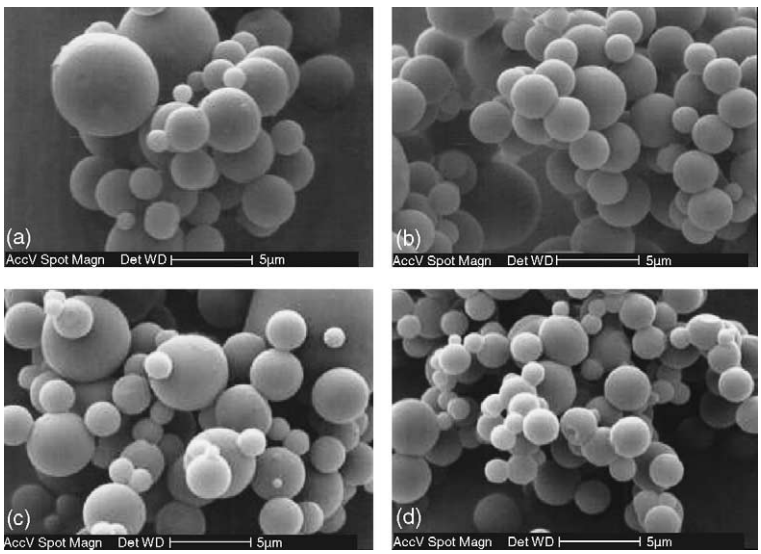

Fig. 1. Scanning electron micrographs of PCL and TPGS–PCL blend microspheres loaded with diphtheria toxoid: (a) A1: PCL alone; (b) A2: PCL + 10% (w/w) TPGS; (c) A3: PCL + 20% (w/w) TPGS; (d) A4: PCL + 40% (w/w) TPGS.

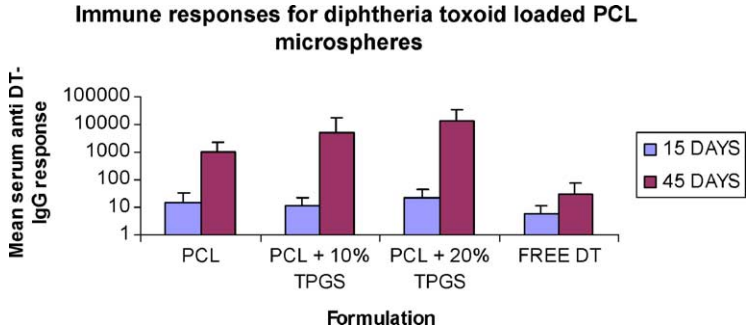

Fig. 2. Specific serum IgG antibody responses for nasally delivered diphtheria toxoid, encapsulated in either PCL alone, TPGS–PCL blended microspheres or in soluble form. BALB/c mice were dosed on day 1 with 10 μg diphtheria toxoid per mouse and boosted on day 28 with 5 μg. Note: $n = 5$ per group; mean ± S.D.

protection of rapidly proliferating cells of the immune system from oxidative damage (Tengerdy and Lacetera, 1991). An important consideration for its use as a mucosal adjuvant is the safety of Vitamin E TPGS, in comparison with alternative absorption enhancers. Due to its safety TPGS has been used to treat Vitamin E deficiency in children and its acute oral LD50 dose is over 7000 mg/kg in adult rats. Clinical studies routinely used dosage regimens of 2256 mg TPGS twice daily and therefore it is anticipated that the concentrations used in our studies were very low and should not cause any tissue damage.

In a number of studies conducted using a hydrophobic anticancer drug paclitaxel, TPGS was used as an emulsifier instead of the traditional and popular PVA during the preparation of polymeric nanoparticles. The resulting particles had a narrower size distribution and superior surface properties. It was found that encapsulation efficiency of the drug in the PLGA nanospheres increased up to 100%.

In conclusion, the PCL microspheres gave better immune responses compared to the free diphtheria toxoid. The immune response for the PCL-TPGS blend microspheres was generally higher than that observed for diphtheria toxoid loaded plain PCL microspheres. The TPGS component may function as a safe mucosal adjuvant, and although the mechanism of action is not clearly understood it could be due to its antioxidant and amphiphilic properties. The mechanism of action of this molecule needs to be explored more thoroughly because compared to known mucosal adjuvants TPGS has an excellent safety profile. It also shows significant potential for use as an adjuvant either alone or in combination with an appropriate delivery system.

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