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Evaluation of synergistic effect of biodegradable polymeric nanoparticles and aluminum based adjuvant for improving vaccine efficacy

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ABSTRACT

Aluminum based adjuvants have been used widely to induce long lasting protective immunity through vaccination. But reported incidences of toxicity and side effects of aluminum have raised concerns regarding their safety in childhood vaccines. The present study demonstrates the synergistic effect of admixture of polylactic acid–polyethylene glycol (PLA–PEG) based biodegradable nanoparticles (NPs) and aluminum phosphate as a potential adjuvant system using tetanus toxoid (TT) as a model antigen. The immunological activity of the admixture formulation was maintained up to 180 days of storage at 5 °C ± 3 °C. Percent adsorption/encapsulation of tetanus toxoid increased to nearly 90% in admixture formulation as compared to 55% in conventional vaccine. Admixture preparation (PLA–PEG–Al 0.2 mg-TT and PLA–Al 0.2 mg-TT) showed 80% and 50% survival respectively, even at 180 days as compared to 30% survival observed in the conventional tetanus vaccine. The present study established the feasibility to formulate a dosage form with improved efficacy and reduced aluminum concentration for vaccination.

1. Introduction

Aluminum based adjuvants have been used extensively to induce long lasting protective immunity through vaccination and billions of doses have been administered over the years (Lindblad, 2004). But, reported incidences of toxicity and side effects of aluminum have raised concerns regarding their safety in childhood vaccines. These effects include minor local reactions such as pain and erythema, a nodule at the site of injection and systemic reactions which may entail fever, malaise, shivering, general aches and headache (Clements and Griffiths, 2002). These are one of the most common reasons for dropout rates, resulting in incomplete immunization and hence susceptibility to various diseases (Aguado, 1993). A small proportion of vaccinated people also suffered from delayed onset of diffuse myalgia, chronic fatigue and cognitive dysfunction. In some persons, a granulomatous lesion called macrophagic myofasciitis (MMF) has also been observed. Clinical symptoms associated with MMF is recently delineated as "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA) (Gherardi and Authier, 2012). Despite of this fact, the choice of adjuvants for human vaccination still reflects a compromise between a requirement for adjuvancity and an acceptable level of side-effects (Clements, 1996). At present, in human vaccinations, aluminum based adjuvants are being used primarily in diphtheria, tetanus, pertussis, hepatitis B, pneumococcal and *Haemophilus influenza* type b vaccines (Baylor et al., 2002). Keeping in view the nascent immune system of children, the number of aluminum based vaccines being given to them may lead to increased side effects. Therefore, it has always been a constant effort and focus to either search for the alternative adjuvants (Gupta, 1998) or to reduce the quantity of aluminum in the vaccines. In this direction, controlled release micro and nanoparticulate formulations based on biodegradable polymers such as poly(lactic acid) and poly(lactic/glycolic) acid (PLGA) and polycaprolactone (PCL) have been investigated (Alonso et al., 1993; Jiang and Schwendeman, 2008; Prashant et al., 2014; Raghuvanshi et al., 2001; Zhao et al., 2014). The efficacy of these formulations was determined only by ELISA in rat or guinea pig model (Johansen et al., 2000; Katare and Panda, 2006; Raghuvanshi et al., 2001), but challenge method recommended by both WHO as well as pharmacopoeia guidelines is a better indicator of the efficacy of

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the vaccine wherein the immunised test animal is directly exposed to the disease causing organism or their product and efficacy is evaluated statistically.

The present study was designed with an aim to evaluate the effectiveness of biodegradable polymeric nanoparticles as adjuvant and also to explore the possibility of a synergistic effect of admixture of polymeric nanoparticles and aluminum at a reduced concentration, as potential candidate for vaccine delivery. The efficacy of various developed formulations was evaluated by ELISA as well as by the single dilution challenge method.

2. Experimental

2.1. Materials

Tetanus toxoid (TT) (3300 Lf/ml) was received as a gift from Human Biological Institute, Hyderabad. Tetanus antitoxin serum (TATS) was obtained from Central Research Institute, Kasauli, India. Polylactic acid (PLA, 72 KDa) was procured from Nature works, Blair, NE, USA, Polyethylene glycol (Mol. Wt. 4000) was supplied by CDH, New Delhi. 4-Dimethyl aminopyridine (DMAP) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) was obtained from Spectrochem, Mumbai. Dichloromethane and acetonitrile were obtained from Merck, Mumbai. Poloxamer F-127, bovine serum albumin (BSA) and anti-goat HRP conjugate were obtained from Sigma–Aldrich, USA. 3,3′,5,5′-Tetramethylbenzidine (TMB) was purchased from Biorad, USA. Ultrapure grade water with a conductivity of 18.2 mΩ cm was used in all experiments. Tetanus toxoid vaccine manufactured by Serum Institute of India Limited (SIIL), Pune, India, Biological E. Limited, Hyderabad, India, Central Research Institute Kasauli, India, Dano Vaccines and Biologicals, Hyderabad, India and Venky Parenteral, Yanam, India were used in the study. Swiss albino mice were supplied by the animal house facility of Central Research Institute, Kasauli, India. The study protocol was approved by the Institute Animal Ethic Committee (IAEC) of Central Research Institute, Kasauli, India. Experimental protocol of immunization is given in Table 1.

2.2. Preparation of vaccine formulations

2.2.1. Preparations of tetanus vaccine having varying concentrations of aluminum

Tetanus vaccine with varying concentrations of aluminum phosphate was prepared by using a method described elsewhere with slight modification (Kumar et al., 2001). Briefly, tetanus antigen (3300 Lf/ml) was diluted with normal saline to get the final concentration of tetanus antigen as 10 Lf/ml and mixed with varying concentrations of aluminum (0.1–1.25 mg/dose). The pH of the formulation was adjusted to 6.2 ± 1.0. The resulting mixture was allowed to mix on a shaker at 250–300 rpm for 3 h, followed by keeping the same for overnight at room temperature. Thereafter, the preparation was stored at 5 °C ± 3°C till further use.

2.2.2. Synthesis and characterization of PLA–PEG block copolymer

PLA–PEG block copolymer was synthesized using polylactic acid (~72 KDa) and polyethylene glycol (4 KDa). In a standard experiment, 0.014 mmol of PLA and PEG was dissolved in 100 ml of dichloromethane in a 250 ml round bottom flask and allowed to stir at 0 °C ± 2 °C. To this solution, 5 ml of 1% *N*,*N*′-dicyclohexylcarbodiimide (DCC) solution was added slowly followed by the addition of 2 ml of 0.1% 4-dimethylaminopyridine (DMAP) and the reaction mixture was stirred for 16 h. The resulting PLA–PEG block copolymer was precipitated with 1:1 mixture of diethyl ether and methanol to remove the unreacted PEG. Synthesized PLA–PEG block copolymer was dried under vacuum and stored at −20 °C till further use. Gel permeation chromatography (GPC) analysis was performed at room temperature using Malvern Viscoteck GPC, USA system with tetrahydrofuran (THF) as mobile phase. The GPC results suggested that a single block of PEG was coupled with a single block of PLA to give a PLA–PEG diblock copolymer Table 2.

2.2.3. Preparations of vaccine loaded nanoparticles (NPs)

PLA and PLA–PEG nanoparticles were prepared by double emulsion solvent evaporation method (Kumar et al., 2014; Tomar et al., 2013). Briefly, the desired polymer (400 mg) was dissolved in 20 ml acetonitrile, followed by addition of concentrated tetanus toxoid with brief sonication in quantity sufficient to get the desired concentration of 10 Lf/ml in the final formulation. The resulting primary emulsion was then added drop wise into a 100 ml aqueous solution comprised of poloxamer F127 (640 mg) in distilled water and stirred at room temperature for overnight to facilitate solvent evaporation and NP stabilization. Nanoparticle suspension was centrifuged at 13,000 rpm for 30 min to get the pellet. After removing the supernatant, NPs pellet was re-suspended in 5 ml of Milli-Q water and freeze dried. Encapsulation efficiency of tetanus toxoid was calculated according to the following formula:

$$\text{Encapsulation effieiency}\% = \frac{\left[\{\text{TT}\}_{\text{total protein}} - \{\text{TT}\}_{\text{protein in supernatant}}\right]}{[\text{TT}]_{\text{total protein}}} \times 100$$

The morphology, particle size distribution and zeta potential (ζ) of the tetanus toxoid encapsulated NPs were determined by scanning electron microscope (SEM; LEO 1455 VP, USA) and nanoparticles tracking analysis (NTA; NS500, Nano sight, UK).

Table 1
Experimental protocol for immunization.

S. No.	Group	Formulation	Dose	Route	Challenge interval (days)
1	GP-1	PLA–TT	0.5 ml	S/C	15,28,56,90,120,150,180
2	GP-2	PLA–PEG–TT	0.5 ml	S/C	15,28,56,90,120,150,180
3	GP-3	Al 0.1 mg-TT	0.5 ml	S/C	15,28,56,90,120,150,180
4	GP-4	Al 0.2 mg-TT	0.5 ml	S/C	15,28,56,90,120,150,180
5	GP-5	Al 0.4 mg-TT	0.5 ml	S/C	15,28,56,90,120,150,180
6	GP-6	Al 0.8 mg-TT	0.5 ml	S/C	15,28,56,90,120,150,180
7	GP-7	Al 1.25 mg-TT	0.5 ml	S/C	15,28,56,90,120,150,180
8	GP-8	PLA–Al 0.2 mg-TT	0.5 ml	S/C	15,28,56,90,120,150,180
9	GP-9	PLA–PEG–Al 0.2 mg-TT	0.5 ml	S/C	15,28,56,90,120,150,180
10	GP-10	Conventional tetanus vaccine	0.5 ml	S/C	15,28,56,90,120,150,180
11	GP-11	Normal saline	0.5 ml	S/C	15,28,56,90,120,150,180

Table 2
Characterization and physicochemical properties of PLA and PLA–PEG block copolymer and nanoparticles.

Sample	GPC[a] of polymeric NPs			Particle size[b] (nm)	Zeta potential[c] (ζ)	Encapsulation efficiency[d] (EE)
	Mn	Mw	Mw/Mn			
PLA	59745	72487	1.213	92.9 ± 2.6	−15.5 ± 1.8	46.3%
PLA–PEG^{4K}	72479	78416	1.082	101.7 ± 2.1	−8.6 ± 1.2	56.8%

[a] Gel permeation chromatography (GPC) of PLA–PEG block copolymers at room temperature using Viscoteck GPC system with THF as mobile phase.
[b] Measured by NTA (NS 500, Nanosight).
[c] Measured by NTA (NS 500, Nanosight).
[d] Encapsulation efficiency expressed as a percentage mean of 3 determinants of tetanus toxoid recovered in NPs compared with theoretical load.