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Effect of functionalization of polymeric nanoparticles incorporated with whole attenuated rabies virus antigen on sustained release and efficacy

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Abstract

Nanovaccines introduced a new dimension to prevent or cure diseases in an efficient and sustained manner. Various polymers have been used for the drug delivery to increase the therapeutic value with minimal side effects. Thus the present study incorporates both nanotechnology and polymers for the drug delivery. Poly(D,L-lactic-co-glycolic acid)-b-poly(ethylene glycol) was incorporated with the rabies whole attenuated viral antigen using double emulsion (W/O/W) method and characterized by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Chitosan-PEG nanoparticles incorporated with the rabies whole attenuated virus antigen (CS-PEG NP-RV Ag.) were prepared using Ionic Gelation method. The CS-PEG NP-RV Ag. was surface modified with biocompatible polymers such as Acacia, Bovine Serum Albumin (BSA), Casein, Ovalbumin and Starch by Ionic Gelation method. The morphology was confirmed by SEM and Transmission Electron Microscopy (TEM). The surface modification was confirmed by Fourier Transform Infrared Spectroscopy (FTIR), Zeta potential. The size distribution of CS-PEG-RV Ag. and surface modified CS-PEG-RV Ag. by respective biocompatible polymers was assessed by Zetasizer. Release profile of both stabilized nanoparticles was carried out by modified centrifugal ultrafiltration method which showed the sustained release pattern of the Rabies Ag. Immune stimulation under in-vitro condition was studied using rosette assay and phagocytosis assay. In-vitro toxicity using human blood and genotoxicity using human blood DNA was also studied to assess the toxicity of the nanoformulations. The results of these studies infer that PLGA-b-PEG nanoparticles, CS-PEG and surface modified CS-PEG nanoparticles may be an efficient nanocarrier for the RV Ag. to elicit immune response sustainably with negligible toxic effect to the human system.

Keywords: Rabies virus; Nanotechnology; Nanovaccines; Biopolymers; Sustained release; Chitosan; PLGA-PEG

1. Introduction

Rabies is the zoonotic disease (a disease transmitted from animals to humans) caused by the rabies virus of genus Lyssavirus, says the World Health Organization (WHO). In several countries, dogs, raccoon dogs, and foxes are considered to be a major vector for rabies [1].

This disease causes acute inflammation on the brain of the infected mammal which leads to death. The disease is ranked endemic on all continents with the highest case incidence in Asia and Africa; it threatens potentially over 3 million people [2].

In 1958, Kissling reported the propagation of the rabies vaccine in hamster cell cultures [3]. This led to the propagation of the virus in cell cultures. Now the virus is generated in chick embryos and attenuated using formaldehyde for vaccination purposes. In the initial stage, the 'vaccination-challenge assay' is widely used for batch release of inactivated rabies vaccine for veterinary use as it attempts to use serological assay [4]. Vaccination against rabies provides immunity to the disease in pre-exposure and postexposure prophylaxis.

Nanovaccines consist of nano-scale based particles attached or formulated with components to which an immune response

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is desired. Nanovaccines are efficient than the conventional vaccines as they induce the humoral and cell-mediated response [5]. With the help of nanotechnology, non-invasive vaccination can be provided effectively such as oral vaccination for invasive vaccination techniques as it elicits cell-mediated immunity and nasal vaccination technique gives rise to systemic humoral, cellular responses, local and distal secretory immune responses thus making mucosal lining less vulnerable to infection [6,7]. Efficient deliveries in the targeted tissues are achieved through nanotechnology [8]. Nanovaccines have evinced the ability for a number of infectious pathogens, including HIV, malaria, tuberculosis, and hepatitis C.

Nanoparticles are the good candidates to act as an adjuvant when compared to the largely used adjuvant, alum [9]. Physicochemical properties, such as particle size, influence the movement of the nanoparticle to travel through the lymphatic and accumulate in lymph node-resident dendritic cells. Nanoparticles used in vaccine formulations increase the immunogenicity and protect it from its loss and thus act as an adjuvant. Nanovaccines are dreamt to formulate vaccines that require needle-less administration, have long shelf life, are less temperature dependent, have sustained immunogenicity and are one-time dose. This study was done with the view that biopolymers have the potential to increase the therapeutic value of drug delivery with minimal side effects. For the preparation of the nano polymer conjugate double emulsion method and ionic gelation method were used. In vitro toxicity using human blood and genome toxicity were also performed.

2. Materials and methods

2.1. Preparation of poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol) nanoparticles incorporated rabies whole attenuated virus antigen

2.1.1. Chemicals and reagents

Poly (D,L-lactic-co-glycolic acid) (50:50) 38,000–54,000 mol. Wt. with terminal carboxyl group (PLGA carboxylate); Dichloromethane (DCM) (both were purchased from Sigma Aldrich). Heterobifunctional PEG (amine-PEG-carboxylate) at molecular weight of 3400 g/mol (NOF Corporation, Tokyo, Japan) is stored in dark at −20 °C and *N*,*N*-diisopropylethylamine (DIEA) (Sigma-Aldrich). Conjugation crosslinkers: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) is stored in dark at −20 °C; *N*-hydroxysuccinimide (NHS) is stored at 4 °C (both from Pierce, Rockford, IL). Poly(vinyl alcohol) (PVA) (88% hydrolyzed, ~22 kDa, Fisher), Methanol (Rankem). All the chemicals were of analytical grade and highly purified.

2.1.2. Synthesis of PLGA-b-PEG polymer

There are two ways to produce PLGA-b-PEG diblock copolymers. The conjugation of PLGA homopolymer with a carboxylate end group using EDC and NHS as conjugation crosslinkers method was preferred in this study over melt or solution copolymerization in the presence of monomethoxy-PEG using stannous octoate as a catalyst due to technical easiness and high yield preferences. The modified method is described below [10].

250 mg of PLGA-carboxylate was first dissolved in 1–2 mL of DCM. NHS (3.0 mg) is dissolved in 4.8 mg of EDC in 1 mL of DCM. The PLGA-carboxylate solution was added to the NHS/EDC solution and gentle stirring was carried out in a magnetic stirrer to form PLGA-NHS. Precipitation of PLGA-NHS was done with 20 mL of methanol washing solvent by centrifugation at 3000 rpm for 10 min and residual EDC/NHS was removed. Washing and centrifugation were done twice to ensure purity of the resultant PLGA-NHS. The PLGA-NHS pellet is dried to remove the residual methanol. After drying PLGA-NHS (246 mg) was dissolved in 4 mL of DCM followed by the addition of amine-PEG-carboxylate and DIEA (1:1) mmol. The mixture solution was incubated under stirring at 600 rpm for 24 hours at room temperature. The resultant PLGA-b-PEG block copolymer was precipitated with methanol wash and centrifuged at 3000 rpm for 10 min to remove the unreacted PEG. The PLGA-b-PEG polymer result was lyophilized.

2.1.3. Synthesis of PLGA-b-PEG NP-rabies whole attenuated viral antigen

The modified double emulsion-solvent evaporation method was used for the preparation of PLGA-b-PEG NP-rabies whole attenuated viral antigen. The rabies whole attenuated viral antigen (100 μL) and PLGA-b-PEG polymer (100 mg) were added with DCM (2 mL) and mixed. Further, the solution was sonicated using Probe sonicator at 20 W for one minute. The emulsion occurred was precipitated with PVA (50 mL of 0.1 w/v). Further, it is sonicated at 20 W, 1 min to form W/O/W emulsion. The emulsion is stirred at 200 rpm, 2 h. The resultant PLGA-b-PEG-rabies whole attenuated viral antigen was centrifuged for 3000 rpm, 15 min and washed with deionized water. It was recovered and suspended in PBS buffer for a short time storage.

2.2. Synthesis of chitosan-PEG NP-rabies whole attenuated viral antigen and functionalization of the conjugate with biocompatible polymers

2.2.1. Chemicals and reagents

Chitosan (CS) (48 kDa, Primex Co, Iceland), cross linker Sodium Tripolyphosphate (STPP); Acetic Acid (Sigma Aldrich), rabies whole attenuated viral antigen with potency ≥2.5 I.U. (Rabipur, Novartis vaccine). Acacia; Bovine Serum Albumin (BSA); Casein; Starch; Ovalbumin (were purchased from Ranken). All the chemicals were of analytical grade and highly pure.

2.2.2. Synthesis of CS NP-PEG-rabies whole attenuated viral antigen

The method followed was the modified method of inotropic gelation using STTP as cross linker [11]. CS (25 mg) was taken and acetic acid (250 μL) was added to it. To this mixture deionized water (75 mL) containing PEG (60 μL) rabies whole attenuated viral antigen was added and stirred (3–4 h). The resultant cloudy suspension was centrifuged at 10,000 rpm for 15 min and sonication was done at 20 W at 5 min to recover CS NP-PEG-rabies whole attenuated viral antigen.