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Development and characterization of a new carrier for vaccine delivery based on calcium-alginate nanoparticles: Safe immunoprotective approach against scorpion envenoming

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ABSTRACT

To enhance humoral defense against diseases, vaccine formulation is routinely prepared to improve immune response. Studies in nanomaterials as a carrier of vaccine delivery are promising and interesting. In this study, attenuated *Androctonus australis hector* (*Aah*) venom and its toxic fraction were encapsulated into different formulations inside calcium–alginate nanoparticles (Ca–Alg Nps), and used as a vaccine delivery system against scorpion envenomation. Ca–Alg Nps were prepared by ionic gelation and characterized. An immunization schedule was undertaken in rabbits in order to study how *Aah* venom entrapped in Ca–Alg Nps might induce protective immunity. Results showed the influence of different parameters on the suitable nanoparticle formation. They also showed no toxicity of free Ca–Alg Nps and a different inflammatory profile depending on the nanovaccine formulations. More interestingly, evaluation of specific IgG titer and IgG1/IgG2a isotype balance revealed a protective effect with the nanoparticles encapsulating the attenuated antigens. Challenge up to 6 LD 50 of native venom, allowed to an important immunoprotection of all immunized rabbits, with no recorded death. Taken together and with respect to the properties of nanoparticles and high immunogenicity, calcium–alginate nanoparticles could be considered as a new promising adjuvant system and a vaccine delivery against scorpion envenomation.

1. Introduction

Nanomedicine is changing the diagnosis of diseases as well as the delivery of biologically active compounds to treat or to prevent some diseases.

Vaccination is needed for a wide range of infectious diseases and for cancer therapy to develop adaptive immune responses in organisms. Vaccines are usually constituted by whole inactivated pathogens, live attenuated viruses, or inactivated toxins and venoms [1–3]. Currently, the necessity to study new adjuvants and delivery systems is the most important question in vaccinology. Several developed vaccines are based on purified subunits, recombinant proteins, or synthetic peptides [4]. These generations of vaccines are safe and with well-defined components. However, these antigens are often poorly immunogenic. Thus, they require the use of adjuvants or delivery systems to induce optimal immune responses [5]. Advances in nanotechnologies have drawn scientists and engineers to investigate nanoparticles to enhance vaccine technology, leading to the birth of "nanovaccinology" [6].

Controlled drug-delivery technology offers numerous benefits compared to conventional therapeutic systems, such as preserving protein bioactivity, extending duration time and decreasing side effects, which consequently increase the therapeutic efficiency [7,8]. This improvement can be done through nanosized systems such as nanoaggregates, nanocapsules and nanospheres up to 1000 nm of diameter size [9–12]. These systems can entrap enzymes, drugs and other compounds such as antigens into the particle's matrix. Nanoaggregates, nanocapsules, or nanospheres are obtained according to specific methods [10]. Nanoaggregates can be described as nanosized colloidal systems in which the antigen is physically dispersed, and can have different morphologies [13,14].

Biodegradable polymeric nanoparticles such as inulin, pullulan, chitosan and calcium–alginate have been developed as a new strategy for vaccine therapy [15,16]. Calcium–alginate and chitosan-based nanoparticles have been widely studied due to their biocompatibility, biodegradability, non toxic nature and their ability to be easily modified into many forms and sizes [17,18]. These

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nanoparticles have been used in the preparation of various vaccines including HBV vaccines [16], Newcastle disease vaccines [19], and DNA vaccines [20].

The emergency caused by scorpion envenomation is considered as a major public health problem in tropical and subtropical regions, due to their lethal effects in human population, especially in children and elderly. The immunotherapy is the only specific treatment used against lethality. The most dangerous scorpion in Algeria is *Androctonus australis hector* (*Aah*), it is the cause of severe accidents after envenomation. Its venom contains neurotoxins with low molecular-weight (~7 kDa). These basic toxins are cross-linked by four disulfide bridges and interact specifically with voltage-dependent sodium channels [21]. The venom and its major toxic fraction "FtoxG50" induce a complex pattern of clinical symptoms affecting the respiratory, nervous and cardiovascular systems, with severe inflammatory response that can lead to the death [22]. Detoxification of venom and its toxic fraction by gamma irradiation with a 2 kGy dose, seems to be a promising tool as it preserve the antigenic and immunogenic properties of antigens for using them in a vaccine approach [2].

In this work, we provide further understanding on the development of the antiscorpionic immunoprotection previously started with our team [5,23]. Native and irradiated *Aah* venom and its toxic fraction FtoxG50-loaded calcium–alginate nanoparticles were synthesized and their physicochemical properties characterized. Their adjuvant quality, non toxic and immunoprotective properties were tested after a medium-term immunization schedule.

2. Materials and methods

2.1. Materials

2.1.1. Chemical reagents

All reagents used in this study are of analytical grade. Low molecular weight of sodium alginate (SA, medium viscosity of 3500 cps; 2% w/v solution), calcium chloride dihydrate ($CaCl_2$), Coomassie brilliant blue G-250, poly-L-lysine (4000–15000 Mw), O-dianisidine hydrochloride, O-phenyldiamine (OPD, 99.5%), (1S, 2S)-1,2-di-1-Naphthyl-ethylenediamine dihydrochloride, trichloroacetic acid and absolute methanol have been purchased from Sigma–Aldrich, St. Louis, USA.

2.1.2. Animals

Female rabbits (2–2.5 kg) and NMRI mice (20 ± 2 g) were obtained from the animal breeding center of the Faculty of Biological Sciences (University of Science and Technology Houari Boumediene, Algiers). Animals were housed in controlled temperature room and received food and water *ad libitum*. The experimental protocols were performed according to the European community rules of ethical committee for animal welfare.

2.1.3. Biological materials

Lyophilized *Androctonus australis hector* venom (Vn) and its toxic fraction FtoxG50 (Fn) were obtained from our team of Laboratory of Cellular and Molecular Biology. Attenuated forms of the venom (V*) and its whole toxic fraction (F*) were irradiated with 2 kGy (765 Gy/h dose rate) γ-irradiation at the Nuclear Research Centre of Algiers (CRNA), Department of Irradiation Techniques.

2.2. Methods

2.2.1. Preparation of calcium–alginate nanoparticles

Ca–Alg Nps were synthesized by ionic gelation method [24]. Calcium chloride solution was added to sodium alginate under constant homogenization rate at 25 °C. The suspension was centrifuged at 15,000 × *g* for 30 min, the pellets of nanoparticles were freeze-dried without using any cryoprotectant. The effect of sodium alginate concentration on the nanoparticle formation was investigated using various concentrations of sodium alginate (0.1, 0.2, 0.3 and 0.4% w/v). Effect of homogenization rate (500, 1500 and 5000 rpm) and homogenization time (60–120 min) were also tested.

2.2.2. Venom and FtoxG50-loaded nanoparticles

Aah venom and FtoxG50-loaded nanoparticles were prepared according to the method of Sarei et al. [25]. Aqueous solution of $CaCl_2$ (0.1% w/v) was added to the sodium alginate solution (0.3% w/v) containing various concentrations of native or attenuated antigens. Nanoparticles were then coated with poly-L-lysine and freeze-dried for further physical–chemical characterizations.

2.2.3. Characterization of calcium–alginate nanoparticles and antigen-loaded nanoparticles

In order to study the morphological difference between free and antigen-loaded Ca–Alg Nps, the irradiated venom-loaded Ca–Alg Nps (Nps–V*) are used as antigen-loaded nanoparticles and free nanoparticles (Nps) as control.

2.2.3.1. Characterization of nanoparticles by Transmission Electron Microscopy (TEM). The morphological characteristics of the nanoparticles were investigated by transmission electron microscope (TEM) (ZEISS LEO 900, Germany). Samples were submitted to copper grids, contrasted with uranyl acetate and dried at room temperature.

2.2.3.2. Characterization of nanoparticles by Scanning Electron Microscopy (SEM). The SEM was performed on a FEI Quanta 650 (FEI, Hillsboro, Oregon). Acetone suspension containing calcium alginate nanoparticles was placed in an aluminum stub. The acetone was then left to evaporate, after that the stub was placed in the sample port of the electron microscope and then analyzed.

2.2.3.3. Characterization of nanoparticles by Fourier Transform Infrared Spectroscopy (FTIR). In order to analyze the interactions between polymers, $CaCl_2$ and antigens on the nanoparticles formation, samples were analyzed by FTIR (Nicolet Magna IR 760, Thermo Fisher Scientific, USA). Dry powder of nanoparticles was mixed with potassium bromide (KBr) and a transparent tablet was formed using an agate mortar. All spectra were recorded at the 4000–400 cm^3 wavelength range.

2.2.3.4. Characterization of the size of nanoparticles. The particle size was measured by Mastersizer 2000 (Malvern Instruments, UK) based on the dynamic light scattering (DLS) technique and the Polydispersity index (PI) indicator of the width of the nanoparticle size distribution, was done. Briefly, a volume of diluted nanoparticle formulations was added to the analyzer and results were collected few minutes later.

2.2.3.5. Encapsulation efficiency and loading capacity. Amount of encapsulated antigen in the Ca–Alg Nps was estimated by calculating the difference between the total amounts of irradiated venom loaded into the nanoparticles and the amount of noncaptured antigens remaining in the supernatant after centrifugation of samples at 12,000 *g* during 30 min, according to Bradford method at 595 nm [26]. The Ca–Alg nanoparticle encapsulation efficiency (EE) and