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Novel Heat-Stable Enterotoxin (STa) Immunogen Based on Cationic Nanoliposomes: Preparation, Characterization and Immunization

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Abstract

The novelties encountered in the field of formulation have provided promising solutions for the problematic vaccine development of hapten molecules. In the current study, the novel preparation of a cationic nanoliposomal immunogen of the heat stable enterotoxin (STa) was reported. STa was produced from clinically ETEC isolate of diarrheic neonatal calves and purified using RP-HPLC. STa was loaded into the cationic vesicles which were characterized for their particle size, surface charge, morphology, STa loading, and stability. The STa loaded cationic nanoliposome was used for mice immunization and the generation of STa antibody was monitored using ELISA. Results displayed the spherical nature of the STa loaded vesicles, their suitable size and homogeneity represented by a particle size of 228.1 nm and a PDI of 0.202. The surface charge of the STa nanoliposomes was +29.9, demonstrating sufficient stability during refrigeration storage. The STa loaded cationic nanoliposome was able to elicit specific STa antibody response, and to confer effective protection against STa challenge in mice. The STa antibody binding titer and neutralization capacity were 10^5 and 10^4 mouse units/ml serum, respectively. The developed system is a one-step procedure, which overcomes the disadvantage of the complexity of generation of the hapten-carrier conjugate. In conclusion, the developed STa-cationic nanoliposomal immunogen is feasible and has the potential to improve effectiveness against ETEC, suggesting its applicability in preventing the harmful effects of ETEC infection in neonatal calves.

Keywords: Nanoliposomes; STa; Immunization; Enterotoxin; Immunogen

Introduction

Enterotoxigenic collibacillosis is a major disease which is principally linked to a diarrheagenic strain of *Escherichia coli*; ETEC, affecting neonatal calves and causing an immense problem to the animal industry [1]. It is also a main causative agent of infectious diarrhea in infants living in developing countries and travellers to these areas, correlating with high death rates [2,3]. Among the enterotoxins produced, the heat stable enterotoxin was found to be the virulence causing agent, as it interacts with the intestinal receptor, guanylyl cyclase C, causing a cascade of secretory diarrheal event [4]. Therefore, the development of a subtle vaccine against ETEC diarrhea is deemed necessary for both human and livestock health as well. However, STa presents a problematic antigen owing to its poor immunogenic nature caused by its small molecular weight which is less than 2 KDa [5,6].

In order to solve the immunogenicity problem of STa, the attempt of its delivery in a nanoparticulated form presents a very promising approach, owing to the potential of nanoparticles in maximizing the immune responses [7-10]. In this regard, nanoliposomes have been considered as one of the utilized antigen carriers, owing to their biocompatible and biodegradable phospholipidic/aqueous nature [11-13], which are capable of encapsulating antigens and adjuvants within the phospholipidic bilayers or in their aqueous core. They were also shown to exhibit different modes of antigen presentation to the immune system, resulting in an appropriate immune response against an encapsulating antigen [14,15].

Therefore, the aim of the current novel study was to explore the potential of cationic nanoliposomes in potentiating the immune response against STa. Cationic liposomes loaded with STa were prepared, characterized and tested for their immunization efficacy.

Materials and Methods

Chemicals, reagents and kits

1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP), Bradford reagent, Freund's adjuvant, Amberlite XAD-2, carbonate buffer, ethidium bromide, methylene blue, trifluoroacetic acid, HPLC grade methanol, chloroform, Dulbecco's modified Eagle's medium (DMEM), [3-(4,5-dimethylthiazol)-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Fetal bovine serum (FBS), Trypsin/EDTA solution were purchased from Sigma Aldrich, Germany. Uranyl acetate-2-hydrate was purchased from Allied Signal, Germany. ELISA washing, blocking and stopping buffers were purchased from Serva Electrophoresis GmbH, Germany. Alkaline phosphatase-conjugated goat anti-mouse IgG antibodies and p-nitrophenyl phosphate (pNPP) Microwell Substrate System were purchased from KPL Company, USA. 244-bp STa and 450-bp K99 primers were purchased from Alpha DNA, Canada. EmeraldAmp GT PCR Master Mix was purchased from Takara Bio Europe, France. Molecular biology grade water and agarose gel were purchased from Lonza, Belgium. Standard synthetic STa peptide was purchased from Pipmec Company, China. Soyabean lecithin (Epikuron 200) was kindly provided by Cargill Company, Germany. A549 cell line was purchased from ATCC (Rockville, USA). Other chemicals and reagents were purchased from local commercial source.

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Purification and characterization of native STa from ETEC

Enterotoxigenic *Escherichia coli* (ETEC) was isolated from diarrheic neonatal calves and identified as STa-producing isolate by detection of STa and K99 genes using 244-bp STa and 450-bp K99 primers. The STa primer sequence is (5'TCCGTGAAACAACATGACGG3', 5'ATAACATCCAGCA CAGGCAG3') and K99 primer sequence is (5'TGCGACTA CCAATGCTTCTG3', 5'TATCCACCATTAGACGGAGC3') [16,17]. A standard PCR protocol was adopted according to Salvadori et al. [17]. Briefly, bacterial strains, grown overnight in nutrient broth at 37°C, were pelleted by centrifugation at 10000 rpm at 4°C for 10 min (Hermle Labortechnik GmBH, Germany), and resuspended in 200 µL of sterile distilled water. Bacteria were lysed by boiling for 10 min; lysates were centrifuged as described above and 200 µL of the supernatant were utilized as the polymerase chain reaction (PCR) template [18]. The reaction was performed in a total volume of 25 µL composed of 12.5 µL of EmeraldAmpGT PCR Master Mix, 1 µL F-STa, 1 µL R-STa, 6.5 µL molecular biology grade water and 4 µL template. Thermal cycler was programmed as following: pre-denature (95°C/5min), denature 95°C/1min, annealing 55°C/1min, extension 72°C/10 min, and final extension 72°C/10 min. Total number of PCR cycles was set for 35 cycles. Amplified DNA products were separated on agarose gel of concentration 1.5% using electrophoresis technique, then stained with ethidium bromide and checked under ultraviolet light.

STa was produced and purified according to Aref and Saeed [19]. Briefly, STa-carrying ETEC isolate was grown at 39°C for 18 h on 500 mL of Casamino acid-yeast extract-salts medium, (pH 8.6). The CAYE–ETEC culture was then transferred into a controlled growth environment of 10-L minimal salt medium using Winpact bioreactor (Major Science Co., USA). STa was purified in 4 sequential steps; first the extraction of cell free filtrate by tangential flow filtration using 0.22-µm cassette filter in Millipore Pellicon System (Merck Millipore), followed by desalting and capture of STa using Amberlite XAD-2 batch adsorption chromatography (BAC). Then, concentration of STa was performed using reverse phase MCI-gel BAC, followed by polishing of STa on C8 column (XBridge, Waters Co., USA) using analytical-semipreparative HPLC system (Breeze 2 HPLC, Waters, US). Specificity of the purified product was tested by suckling mouse assay (SMA) according to Giannella [20]. Briefly, a sample from the HPLC purified fraction was serially diluted at ten-fold dilution and 10 µL of 0.2% w/v Evans blue was added per mL. 100-µL sample was orally inoculated in Swiss Albino suckling mice (2-3 days old) using a 1 mL syringe and a 20-µm-diameter polyethylene tube. Mice were euthanized by carbon dioxide in a CO_2 chamber 2 h post inoculation and the intestine was removed and weighed. The intestinal weight to remaining body weight ratio was determined. One unit of ST activity (one mouse unit) is defined as the minimal amount of toxin that produces an intestinal weight/ remaining body weight ratio of 0.083 (cut off value of positive SMA). The identity of this fraction was tested against a standard synthetic STa peptide using RP-HPLC. STa peptide was eluted with the following gradient program: initially (100% A and 0% B), 0-5 min (70% A and 30% B) and 5-60 min (20% A and 80% B) and 60-80 min (100% A and 0% B) at a flow rate of 2 mL/min, solvent A being 0.1% TFA in H_2O and solvent B being 0.1% TFA in 80% methanol.

Preparation of cationic nanoliposomes loaded with STa

Cationic STa liposomes were prepared using the thin film hydration method [21,22]. Briefly, 90 mg soyabean lecithin and 10 mg cationic lipid (DOTAP) were dissolved in 4 mL chloroform and 2 mL methanol, which were evaporated using a rotary evaporator (Janke and Kunkel, Germany) at 50°C to form a thin lipid film on the wall of the flask. The thin lipid film was hydrated with 5 mL acetate buffer pH 4.5 yielding a vesicular dispersion. The cationic liposomal dispersion was then rotated at 150 rpm for 1 h, followed by extrusion via carbonate filter of a pore size 200 nm (Nucleopore Millipore, Netherlands) using a manual extruder (LiposoFast-Basic, Avestin Europe GmbH) for five cycles. The obtained nanoliposomes were left to mature in the refrigerator overnight before conduction of liposomal characterization. The STa loaded nanoliposomes were prepared using the same aforementioned steps, except that 2.5 mg native STa were dissolved in the acetate buffer used for lipid film hydration.

Measurement of the particle size and zeta potential of the prepared cationic nanoliposomes

The particle size, polydispersity index and zeta potential of the prepared plain and STa loaded nanoliposomes were measured using the Zetasizer (Nano ZS, Malvern Instruments Ltd., Worcestershire, UK) [12].

Morphology of the cationic STa loaded nanoliposomes

The morphological features of the cationic STa loaded nanoliposomes were examined using transmission electron microscopy (Jeol, JEM 1010, Tokyo, Japan), after allowing the vesicular sample to be adsorbed onto a carbon coated grid and become negatively stained using 2% uranyl acetate solution [12,22,23].

Measurement of STa loading within the cationic nanoliposomes

In order to calculate the conjugated amount of native STa within the cationic nanoliposomes, centrifugation of the formula was performed at 5000 rpm for 30 min using vivaspin tubes (Vivaspin 2 centrifugal concentrator, PES membrane, MWCO 300 kDa, Sartorius Stedim, Nieuwegein, Netherlands). An aliquot of the supernatant was diluted with distilled water, and measured spectrophotometrically (Jenway, UK) for STa content using the Bradford assay at 600 nm [24].

Measurement of the stability of the STa cationic nanoliposomes

The stability of the prepared STa cationic nanoliposomal formula was assessed by re-measuring the STa loading, particle size, and zeta potential after 3 months storage in the refrigerator, as an index of stability [11,22].

Cytotoxicity evaluation of STa cationic nanoliposomes

Cytotoxicity of STa cationic nanoliposomes was determined by MTT assay on lung epithelial cell line (A549) according to Mosmann [25]. The assay is based on the ability of living cells to reduce a water-soluble yellow dye, MTT, to a purple colored water-insoluble formazan (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) product by mitochondrial enzyme succinate dehydrogenase. In this assay, lung epithelial cells (A549) were maintained in DMEM supplemented with 10% FBS and Streptomycin/Penicillin antibiotics (100 µg/mL) in a humidified air atmosphere (5% CO_2, 95% RH, 37°C). The cells were passage every 2-4 days and split at a ratio 1:6 to 1:3. Cells between 30 through 35 passages were used for the experiment. At 75% confluency, the cells were removed from the flask by trypsinization (trypsin-EDTA solution), counted under the inverted microscope using a haemocytometer and properly diluted with the complete growth medium. The cells were then seeded at a density of 10^4 cells/well in 96-well microtiter plates and placed in a 5% CO_2 incubator for 24 h.

Different concentrations of liposomal formulation containing equivalent amounts of native STa ranging 0.5-32 µg/mL were prepared in DMEM along with a blank liposomal suspension containing equivalent lipids amount. STa solutions prepared in DMEM were tested along the liposomal formulations.

The culture medium from each well was aspirated and replaced by 100 µl of medium (serum free) containing the liposomal formula followed by incubation for 24 h. Thereafter, the media was removed and cells were rinsed with 100 µl PBS. An amount of 100 µl of fresh and complete culture medium was dispensed in each well and incubated for 20 h to stabilize the cells. Twenty µl MTT solution (5 mg/ml in PBS, pH 7.4) was added to cells followed by incubation for 3 h. The medium was aspirated and the formazan crystals were solubilized with 200 µl dimethyl sulfoxide for 15 min. The absorbance was read at 570 nm on a microplate reader. Cell viability was determined as a percentage of the negative control (untreated cells) according to the following equation:

$$\% \text{ cell viability} = [A(\text{test})/A(\text{control})] \times 100$$

Where A (test) is the absorbance obtained for each of the concentrations of the test substance, A (control)is the absorbance obtained for untreated cells (incubated with medium only). The latter reading was assumed to correspond to 100% cell viability. The assay was performed on three occasions with six replicates at each concentration of test substance in each instance.

Immunization and generation of STa polyclonal antibodies

Eight-week-old Swiss male mouse (n=7) weighing on average 35 g, were obtained from the animal house at Assiut University. Mice were kept under standard housing condition and checked on a daily basis for their health status. All animal procedures performed in this study were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines, and have been approved by the IACUC of Ain Shams and Assiut University (approval number REC-ASU 17). Blood samples were collected from orbital sinus [26] using microhematocrit tube from each mouse before conducting immunization to obtain a baseline data.

Immunization procedure of mice was conducted according to Muderhwa, et al. [27] and Richards et al. [28]. Briefly, 1 mL of STa nanoliposomes (containing an amount of STa equivalent to 80 µg) mixed with 1 mL of Freund's complete adjuvant using two sterile luer-lock glass syringes until a stable emulsion was obtained. The mouse was inoculated with 200 µL of the described emulsion (8 µg STa per mouse) at 2 different subcutaneous sites (dorsum and inguinal area) using 25-gauge needle. Each mouse was similarly inoculated with a booster dose of STa- nanoliposome mixed with 1 mL of Freund's incomplete adjuvant at 10-day intervals. Over the course of the 6-weeks immunization schedule, each mouse received a total of four doses of STa- nanoliposomal formulation. The last two doses were given subcutaneously and intra-peritoneally. Mice were bled 10 days after boosting immunization. Sera were separated and tested for STa antibody binding titer and neutralization capacity using indirect ELISA and SMA, respectively.

Indirect ELISA for detecting and monitoring STa antibodies

An indirect antibody-capture ELISA was performed to detect the presence of STa antibodies. Principally, STa antigen was bound to a solid phase, and allowed to react with antibody-containing samples [29] in order to test and monitor the presence of STa antibodies in mouse sera. Briefly, 96-well microtiter plate was coated with 2.5 µg STa/100 µL 0.05 M carbonate buffer, pH 9.6 and incubated overnight at 4°C. Plates were washed four times with 0.01M PBS-0.05% Tween-20 as washing buffer and blotted dry. One hundred µL of 0.5% BSA-0.01 M PBS-0.1% Tween-20 serving as blocking buffer was added to each well to block nonspecific binding sites, followed by incubation at 37°C for 30 min. The plate was then washed with washing buffer and blotted dry. Serum samples were tested for STa antibody at ten-fold dilution (10^{-2}-10^{-6}). One hundred µl from each dilution was added to the reaction plat in duplicate and incubated at 37°C for 45 min. Plate was washed and blotted dry as previously described and 100 L of 1000-fold diluted alkaline phosphatase-conjugated goat anti-mouse IgG antibodies was added to each well and incubated at 37°C for 45 min. A 100 µL of freshly prepared substrate solution (*p*-nitrophenyl phosphate Microwell Substrate System) was added to each well after washing and blotting. The reaction was allowed to develop for 30 min at 37°C. The reaction was stopped by 100 µL of 5% EDTA serving as stop buffer. The developed color was read at 405 nm with an ELISA plate reader (Stat Fax 2100, Awareness, US). Reciprocal value of the maximal dilution of serum having an O.D (Abs405) greater than the mean of Abs405 of STa antibody negative (baseline serum sample) sera+2 s.d (x+2 s.d), was reported as antibody end titer for each tested serum sample.

STa- antibody neutralization capacity assay

STa- antibody neutralization capacity assay was based on SMA and performed according to Aref and Saeed, [30]. Briefly, three aliquots of pooled serum sample (50 µL each) were incubated with 12.5, 25 and 50 µL of STa stock solution (20 MUs/µL) at 37°C for 2 h. The contents of each tube were brought to a final volume of 0.5 mL with PBS before challenging. Three suckling mice were used for each aliquot. In addition, two controls were included: first one had 12.5 µL STa (20 MUs/µl) mixed with 50 µL of PBS and the second one had 12.5 µL STa (20 MUs/µl) mixed with 50 µL of baseline serum. Neutralization capacity is defined as the total MUs of STa that were neutralized per one mL serum.

Statistical analysis

The mean values and standard error were calculated for all experiments carried out in triplicate. Values were tested for their statistical significance using ANOVA followed by Tukey Kramer post hoc test at $p \leq 0.05$ using Graph pad Instat software version 3.06 (La Jolla, CA, USA).

Results

Purification and characterization of native STa from ETEC

HPLC-purified STa was successfully obtained using the aforementioned protocol. Figure 1 displays the detected STa (244bp) and K99+pilus (450 bp) genes in ETEC strain, and Figures 2A and B display the RP-HPLC elution profiles of STa peptide on the preparative C8 column and the elution profiles of the biologically active peaks from preparative runs on the analytical C8 column, respectively. Regarding STa biological activity using SMA, the dilutions of 1/100 and 1/10,000 were positive and induced intestinal weight/carcass ratio of 0.169 ± 0.03 and 0.095 ± 0.01, respectively as shown in Figures 3A and B.

Preparation and characterization of STa cationic nanoliposomal formulations

Cationic liposomes formulated with soyabean lecithin/DOTAP and hydrated using acetate buffer pH 4.5 were successfully prepared. Upon comparing the properties of plain and STa loaded cationic liposomes,

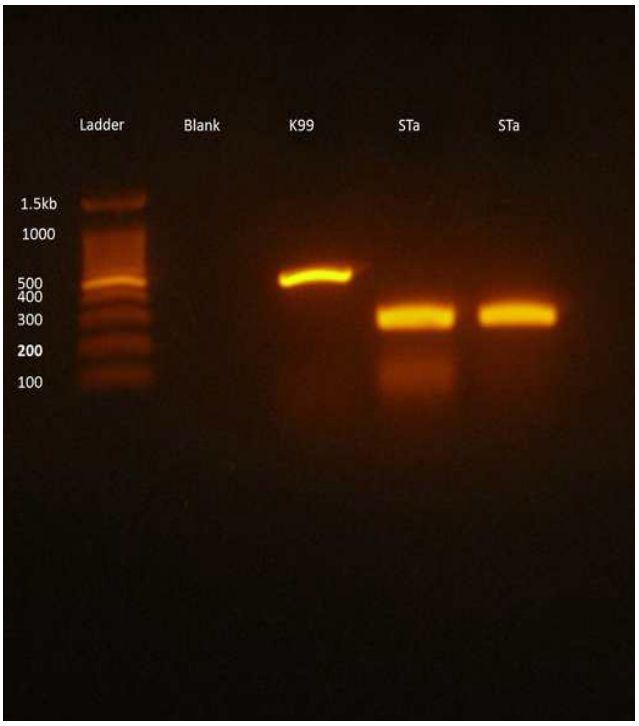

Figure 1: Detection of heat-stable enterotoxin (244bp) & K99+ pilus (450bp) genes in ETEC strain.

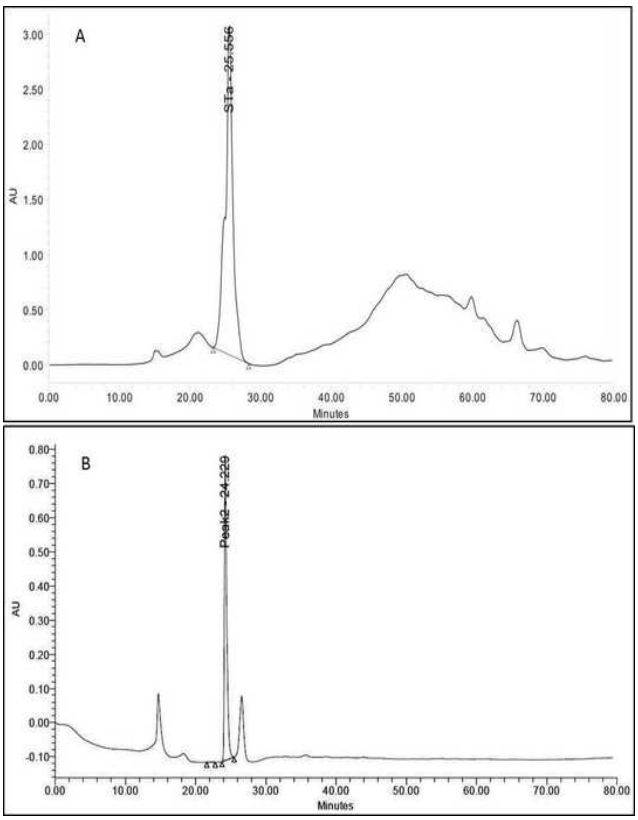

Figure 2: A) RP-HPLC elution profile of STa-peptide on C8 preparative column. **B)** RP-HPLC elution profile of STa-peptide on C8 analytic column.

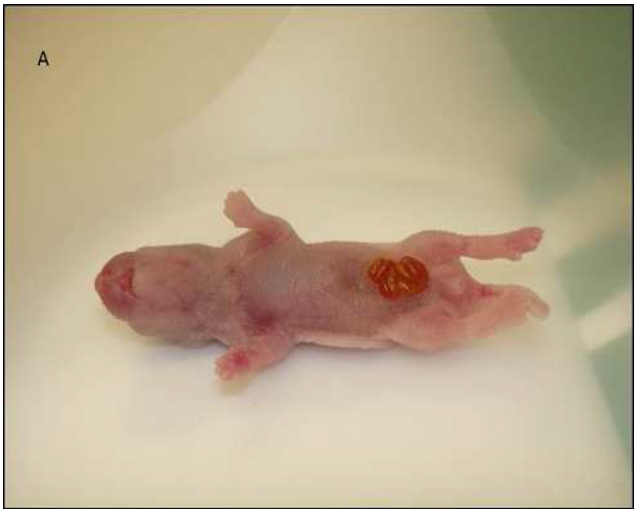

Figure 3A: Negative SMA showing intestinal weight/carcass ratio of <0.083.

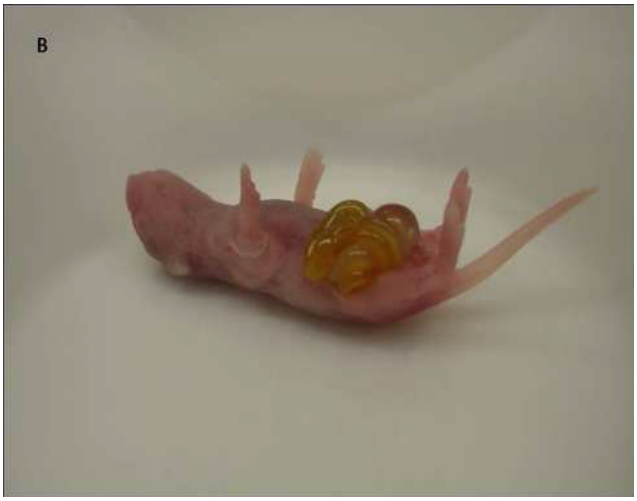

Figure 3B: Positive SMA showing intestinal weight/carcass ratio of 0.169 ± 0.03.

it was found that the former exhibited a particle size of 234.4 ± 5.64 nm, a PDI value of 0.347 ± 0.012, and a surface charge of +36.1 ± 0.99 mV. On the other hand, the STa loaded cationic liposomes displayed a particle size of 228.1 ± 1.41 nm which was non-significantly different from that of plain liposomes ($P>0.05$), a PDI value of 0.202 ± 0.004, and a zeta potential value of +29.9 ± 1.36 mV, which was statistically lower than that of plain liposomes ($P<0.05$). Regarding the stability of STa cationic nanoliposomes after storage for three months, there was non-statistically significant change in the particle size, PDI or zeta potential values.

The morphological characterization of STa cationic nanoliposomes using TEM (Figure 4) displayed their sealed spherical vesicular nature with a particle size concurring with that obtained with the Zetasizer device. STa loading within cationic liposomes was determined using Bradford assay. STa was found to be loaded onto the liposomes with an entrapment efficiency of 12.56% ± 4.2.

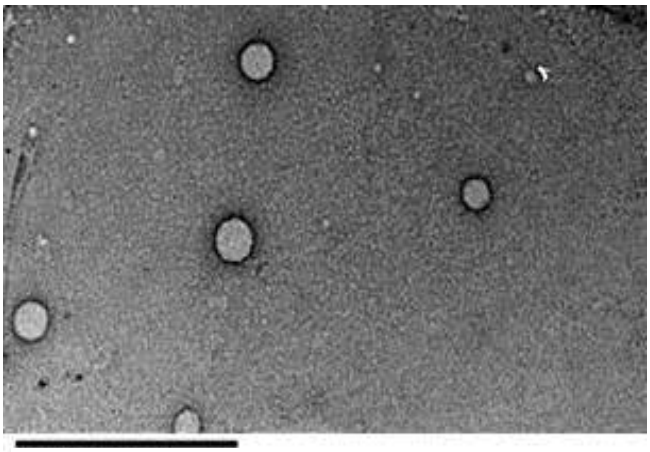

Figure 4: Transmission electron micrograph of STa-loaded cationic nanoliposomes, taken at a magnification of 12000X.

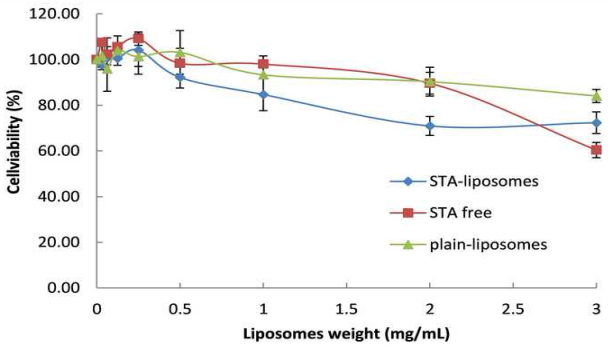

Figure 5: A549 cell viability measured by MTT cytotoxicity assay after exposure to increasing concentrations of STA, STA loaded liposomal suspension and equivalent concentration of plain liposomal suspension. n=3 experiments, six replicates per experiment at each test concentration.

Cytotoxicity evaluation of STa cationic nanoliposomes

In order to assess the safety of STa, blank nanoliposome and STa-nanoliposome, MTT assay was performed. As obvious from the results in Figure 5, STa, blank nanoliposome and STa-nanoliposome conjugate displayed considerable safety on the cells, as can be inferred from their high viability percentages, suggesting its safety for potential animal immunization. Worthy to note that the encapsulation of STa within liposomes significantly reduced its high-concentration toxicity, as can be denoted by the significantly higher cell viability of the STa loaded liposomes (72.36% ± 4.71) compared to mere STa (60.38% ± 3.36) at 48 ug/ml STa concentration.

STa antibody binding assay

No antibody specific response against STa was detected in the baseline serum samples. Thirty one days post-immunization, STa-specific IgG antibody responses were detected in the sera of immunized animals. The STa antibody response dramatically increased over time and attained the highest antibody response between day 41 and 61. Figure 6 showed the pattern of immune response against STa nanoliposome at different time points of immunization. Based on the aforementioned calculation of antibody titer, immunized animals displayed substantially high STa antibody titer (10^5).

Neutralization of STa enterotoxicity

Sera from immunized mouse were tested for the presence of STa-antibody neutralization using an in vivo neutralization assay in the suckling mouse model [30]. Tested sera showed neutralizing activity against STa enterotoxin in STa-challenged suckling mice (SMA<0.083, Figure 7). Based on the aforementioned calculation of neutralization capacity, one ml sera of immunized animals was empirically able to neutralize 10,000 mouse units.

Discussion

Given the fact the STa is poor immunogenic (<2000 Daltons) [5,6], hence the first step toward triggering an appropriate immune response against STa is to couple it to an appropriate carrier. Several strategies have been investigated to make STa immunogenic either through chemical conjugation [6,31-33] or recombinant fusion protein [7,34-38]. These approaches have been assessed based on antigenicity of the final product or complexity of the procedure. Recombinant fusion proteins display weak magnitude and durability of immune responses [7]. Chemical conjugations can display a considerable STa antigenicity [6], however the formation of such conjugates is not always reproducible due to inconsistent hapten-protein stoichiometries, resulting in large variations in the generation of the desired antibodies [39].

In the present study, we investigated a new approach to make STa immunogenic using nanotech and new formula approach. To the best of our knowledge, formulation of immunogenic STa based-nanoliposome has not been investigated before. To do so, cationic liposomes formulated with soyabean lecithin/DOTAP and hydrated using acetate buffer pH 4.5 were successfully prepared. The choice of the acidic buffer for the preparation of the liposomal formulations was based on the fact that STa has an isoelectric point of 3.98 [40] hence, it would be maximally stabilized when hydrated with a buffer close to this pH range, in addition to bearing a negative charge in order to be able to electrostatically bind to the positively charged cationic liposomes [41].

The nanometer size (234.4 ± 5.64 nm) and the low polydispersity index (0.347 ± 0.012) exhibited by the liposomes formulation could be ascribed to the extrusion process which led to the attainment of a liposomal population of narrow particle size distribution. The positive charge (+36.1 ± 0.99 mV) is attributed to the incorporation of DOTAP as a cationic lipid within the formulation. On the other hand, the STa loaded cationic liposomes displayed a particle size of 228.1 ± 1.41 nm which was non-significantly different from that of plain liposomes (P>0.05). They also displayed a significantly lower PDI value of 0.202 ± 0.004 suggesting better sample homogeneity. Moreover, zeta potential value of STa loaded cationic liposomes (+29.9 ± 1.36 mV) was statistically lower than that of plain liposomes (P<0.05), suggesting a possible electrostatic binding between STa and the cationic lipids at the utilized pH, leading to a decrease in the magnitude of positive charge on the vesicles. Overall, such a positively charged liposome might induce a better interaction between STa peptide and a thymus derived lymphocyte (T cell) [42]. The obtained particle size value for STa loaded cationic nanoliposomes was reported to be advantageous for immunogen delivery, as it was shown to increase the persistence of antigens at injection sites, allow better transit to the draining lymph nodes, and elicit IL-12 in macrophages [43].

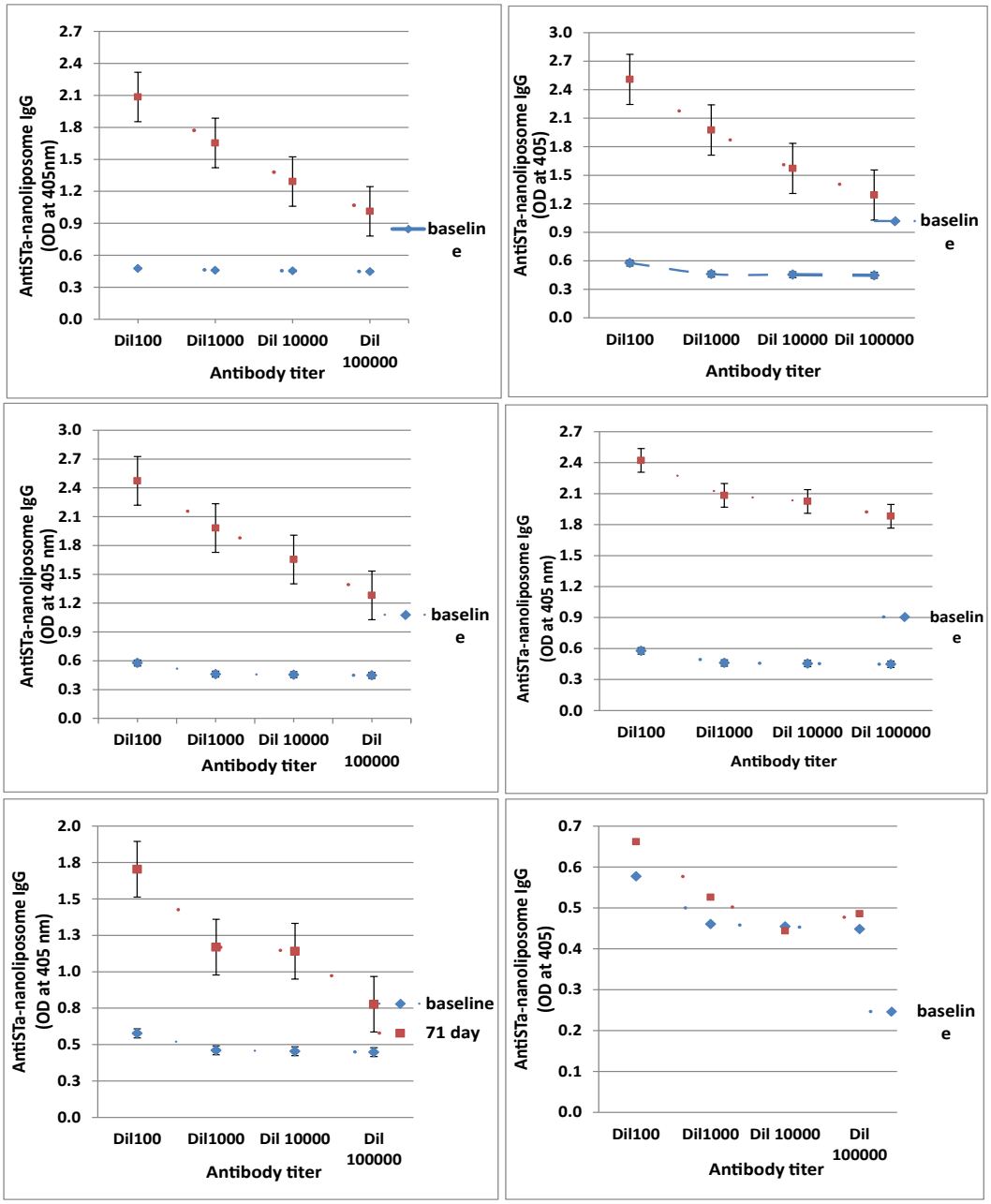

Figure 6: ELISA OD and antibody titer of antiSTa-nanoliposme IgG at 31, 41, 51, 61, 71 and 81 days post immunization. Data presented as Mean ± SEM.

Regarding the stability of STa cationic nanoliposomes after storage for three months, there was non-statistically significant change in either the particle size, PDI or zeta potential values, suggesting the subtle retaining of their physicochemical properties during refrigeration storage. This stability might be attributed to the charged nature of the vesicles, which allowed the vesicles to resist aggregation and fusion [44,45] Furthermore, being in the range of +30 mV, the vesicular dispersions were considered to be adequately stable by creating sufficient repulsion between the particles [46].

STa loading within cationic liposomes with STa entrapment efficiency of 12.56% ± 4.2, strongly suggested the electrostatic and physical incorporation of STa onto cationic lipids.

Potentiation of STa immunogenicity through incorporation in a novel delivery system was successfully designed. As can be inferred from the results, the immunization of mice using STa- nanoliposomes led to the production of specific neutralizing and binding antibodies against native STa. The use of nanoliposomes as a vehicle of STa peptide proved to be an efficient strategy to elicit an excellent humoral response against STa. Furthermore, the described method of preparation of STa-cationic nanoliposomal immunogen is a one-step procedure, which overcomes the disadvantage of the complexity of generation of the carrier conjugate.

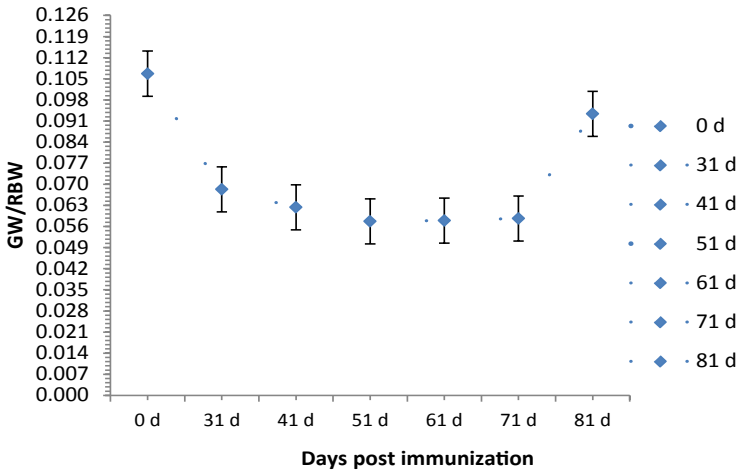

Figure 7: Time-course evaluation of STa antibody neutralization bioassay.

Conclusion

In the present study, we showed that STa cationic nanoliposomes were able to elicit a strong antibody response, as well as confer effective protection against STa challenge in mice. Therefore STa loaded cationic nanoliposome may have the potential to improve vaccine effectiveness against ETEC infection. In short, this study describes the generation of an ETEC STa nanoliposomal immunogen candidate, which is capable of inducing a strong systemic antigen-specific antibody responses in mice, for prevention of ETEC infections.

Conflict of Interest

Authors declare no potential conflicts of interest.

Acknowledgments

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