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Investigation of Niosomes for use as brucellosis vaccine

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Abstract

Background: Niosomes are a promising new drug delivery system that utilizes vesicles to provide sustained, controlled, and targeted delivery of drugs. Not only are they effective for delivering drugs, but they also show potential as vaccine candidates. With their unique properties and capabilities, niosomes represent a valuable tool for advancing the field of drug delivery.

Objectives: The primary objective of this study was to analyze the host's immunogenic response to niosomes loaded with a chimeric protein as a potential brucellosis vaccine candidate.

Methods: In 2020, 50 BALB/c mice were used in this experimental investigation to assess the immunogenic response to niosomes containing a chimeric protein as a potential brucellosis vaccine candidate. The mice were randomly assigned to different groups for injection, oral, and inhalation administration of the vaccine. Brucella strains were obtained from the Razi Vaccine and Serum Institute, and microbiological studies were conducted using the microbial collection from the Pasteur Institute of Iran. Luria-Bertani liquid and solid culture medium from Merck, Germany; Brucella agar culture medium from Gibco, USA; and Kanamycin antibiotic from Roche, Germany, were used during the study.

Results: The study showed promising results, indicating that niosomes are an effective and safe approach in the design of a Brucella vaccine. The immunogenic response in the laboratory mice was found to be satisfactory, suggesting that this type of vaccine could be a suitable candidate for protection against brucellosis.

Conclusion: Niosomes have emerged as a promising drug delivery system for vaccines. They offer controlled, sustained, and targeted delivery of vaccines, which is essential for effective and safe immunization. Overall, these findings suggest that niosomes hold great potential in vaccine development and could play a crucial role in the fight against various diseases.

Keywords: Brucella, Chimeric Protein, Vaccine.

Introduction

The use of noisome as an agent for the cosmetic industry was first introduced in the 1970s and later explored for potential applications in drug delivery.[1,2] Among various vesicles in pharmaceutical systems, niosomes have gained significant attention for drug delivery.[3] Niosomes are vesicles composed of nonionic surfactants,[4] which are capable of delivering both hydrophilic and therapeutic drugs. The preparation of niosomes often involves the use of cholesterol and its derivatives.[5-7] Niosomes are formed through the self-assembly of nonionic surfactants in an aqueous medium, forming concentric bilayer vesicles with a liposome-like structure.[8]

Malta fever, also known as brucellosis, is a prevalent zoonotic infection that can be transmitted between humans and animals.[9] The causative agent of this disease is intracellular gram-negative coccobacilli. It can be transmitted through consumption of contaminated unpasteurized animal products, contact with tissues and fluids of infected animals, or inhalation of contaminated

aerosol particles.[10-12] The prevalence of Malta fever exceeds 10 cases per 100,000 people in some countries.[13,14]

The World Health Organization (WHO) has developed general strategies, including the Mediterranean Zoonoses Control Program, to control Malta fever. These strategies are as follows:

i) Preventing the spread of the disease among animals by monitoring herds and areas free of Malta fever.

ii) Identifying infected animals using diagnostic tests and eliminating them by implementing slaughter programs to produce herds and areas free from Malta fever.

iii) Reducing disease outbreaks by implementing extensive vaccination programs.[15,16]

At present, there is no safe and effective human vaccine available to protect against Malta fever. However, in recent years, research groups have conducted extensive studies to develop a vaccine against Brucella. Based on existing cases, it appears that an antigen capable of stimulating both the humoral and cellular immune systems would be ideal and highly desirable.

Objectives

The aim of this study was to assess the host response to niosomes harboring chimeric proteins as a potential brucellosis vaccine candidate.

Methods

Microbial strains

This experimental study was conducted in 2020, utilizing 50 Balb/c mice that were randomly divided into various injection, oral, and inhalation groups. The live-attenuated B. abortus RB51 and B. melitensis Rev1 vaccines were obtained from the Razi Serum Institute.

Bacterial culture media

MERCK, Germany, prepared the LB liquid and solid culture media, while Gibco, USA, provided the Brucella agar culture medium.

Antibodies and protein substances

The anti-mouse IgG-HRP conjugate was obtained from Sigma, while bovine serum albumin (BSA) was sourced from Merck, Germany.

Biological products

Freund's complete adjuvant and Freund's incomplete adjuvant were acquired from Sigma. A protease inhibitor was procured from Roche, Germany, and sodium azide was obtained from Merck, Germany. Sigma manufactured the TMB substrate for the ELISA test, which was then utilized according to the manufacturer's instructions. Roche provided the POD substrate for the western blot assay.

Chromatography column and ELISA plate

The Ni-NTA agarose resin column for recombinant protein purification was purchased from QIAGENE. ELISA plates were obtained from NUNC.

Gene synthesis in expression cells

Because of its ability to synthesize the T7 promoter-stimulating protein on the plasmid pET28a (+), the E. coli expression strain serves as an appropriate expression host, promoting the production of the desired gene in this bacterium.

Measuring protein concentration by spectrophotometric method (Bradford method)

To ascertain the protein concentration, we adopted the Bradford method. Essentially, this method relies on the binding affinity of the coomassie dye to proteins under an acidic pH.

Investigating the recombinant proteins produced in the host strain in terms of solubility or nisome formation

For the first four hours following induction, 50 ml of samples were collected at one-hour intervals to determine the solubility or nisome development of the recombinant proteins produced in the host strain. Before sedimentation, the bacteria were rinsed with a saline-phosphate buffer and treated to the effects of ultrasonic waves. The collected samples were then injected into the mouse peritoneum with nisome at precise intervals of 0, 14, and 28 days, and another 50 ml sample was collected for further examination at the same time frame after induction and sedimentation.

To stimulate an immune response in laboratory animals, female BALB/c mice aged 4-6 weeks were subcutaneously (s.c.) vaccinated with recombinant protein. Blood samples

were collected on days 10, 24, and 38 to investigate immune responses in the vaccinated mice by the indirect ELISA method. The amount of IgG1, IgG2a, and IgG antibodies against recombinant proteins was determined for analysis.

One month after the final injection, spleen cells were cultivated, and cytokine production was evaluated through the use of an R&D kit for cytokine measurement. By assessing the proliferation power of spleen cells with yellow tetrazolium salts, the MTT method was used to measure the proliferation rate of cells in the culture medium.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (code: IR.BMSU.REC.1397.373). All colleagues had completed the course and unit of working with laboratory animals. The minimum number of laboratory animal was considered to obtain valid results.

Statistical analysis

Data related to each experiment was analyzed with SPSS (version 20.0, SPSS Inc, Chicago, IL, USA). The mean and dispersion indices were calculated for the data of each group. Each group was compared with all other groups through One-way ANOVA and Games-Howell and LSD tests. Comparison of the data of the groups was done two by two using the non-parametric method and the comparison of two non-dependent variables. A "P-value" less than 0.05 was considered significant.

Results

Expression, purification and western blotting of recombinant chimeric protein

The recombinant chimeric proteins were successfully expressed and found to be present in the solution state, as depicted in Figure 1. The proteins were extracted and purified through the use of nickel resins.

Type of injection and blood sampling time in ELISA

It was observed that the absorption rate decreased as the dilution of the sample increased. Furthermore, in antigen-free conditions, the average absorbed dose decreased as the dilution increased on days 10 and 24. The results for dermal injection demonstrated that the average amount of absorption decreased as the dilution increased on days 10, 24, and 38. Similarly, with oral injection, the average absorbed dose diminished with time and increasing dilution. Comparably, for inhalation and landing injections, the absorbed dose decreased with dilution and time. The antibody titer was measured through ELISA, and the results are depicted in Figures 2–5.

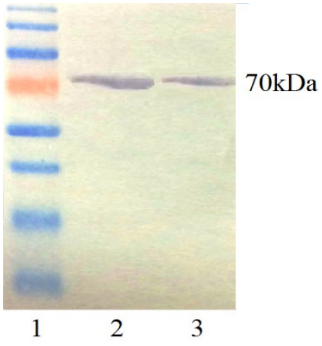

Figure 1. The result of western blotting with anti-histidine antibody

Discussion

The drug composition within niosomes plays a crucial role in precisely targeting the drug to its intended destination. Niosomes have emerged as a promising drug delivery technology owing to their ability to encapsulate various drugs within a versatile and multi-environmental structure. The diverse composition of niosomes makes them a potential candidate for medicinal use.

Rathee et al. conducted a study to assess the efficacy of niosomes as drug carriers for encapsulating Toll-7 receptor agonists and IDO inhibitors. In this study, 1-benzyl-2-butyl-1H-imidazo[4,5-c]quinoline-4-amine (BBIQ) and 1-methyl-D-tryptophan (D-1MT/Indoximod) were encapsulated within the niosomes, and their size and morphology were determined using particle size and microscopic analysis. The study observed that the empty and drug-filled niosomes demonstrated high entrapment efficiency (>90%). Release behavior and kinetic modeling were employed to analyze the drugs' release from the niosomes, while the fluorescence probe immersion method helped determine the distribution coefficient and location of the drugs within the niosomes.

According to the findings of the study, niosomes have promising potential as carriers for the simultaneous

delivery of TLR7 agonists and IDO inhibitors, making them a suitable candidate for cancer vaccine research.[17]

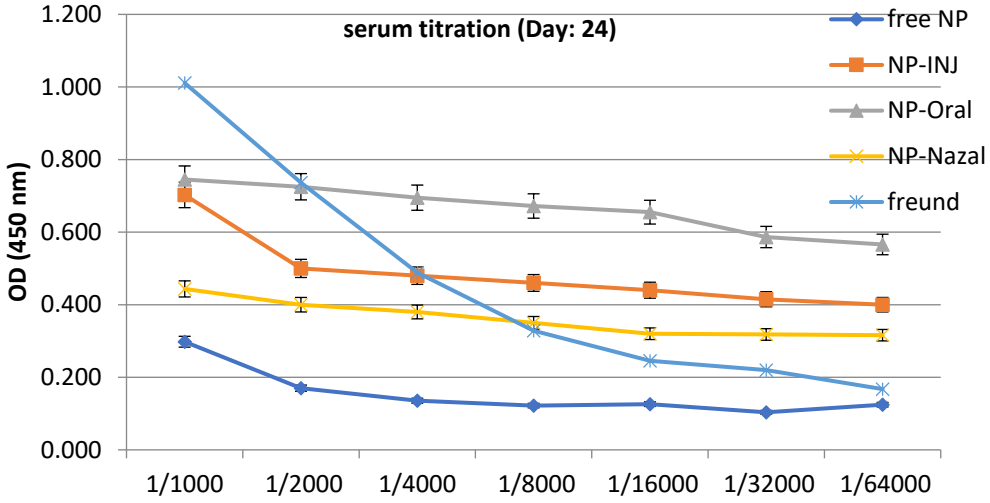

Figure 2. Day 24 titration plot

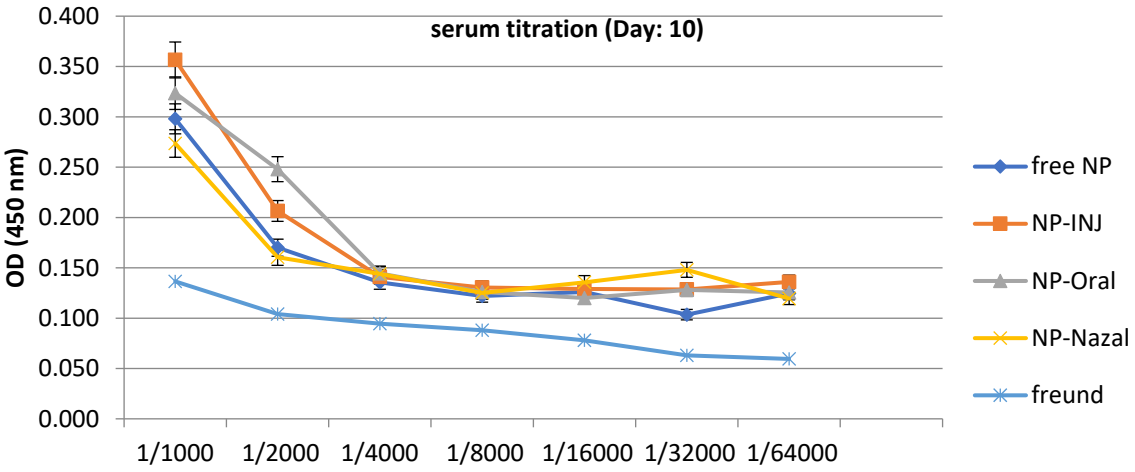

Figure 3. Day 10 titration plot

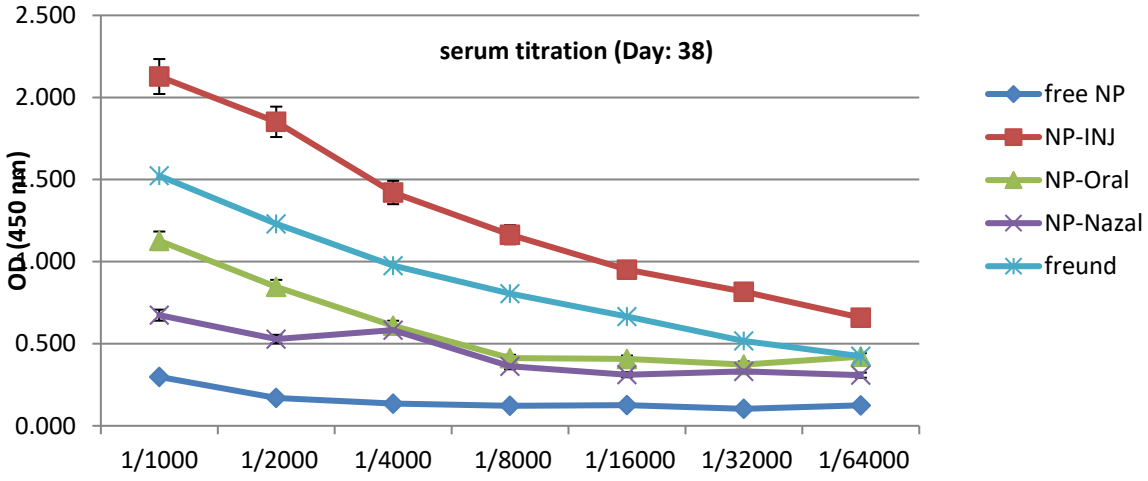

Figure 4. Day 38 titration plot

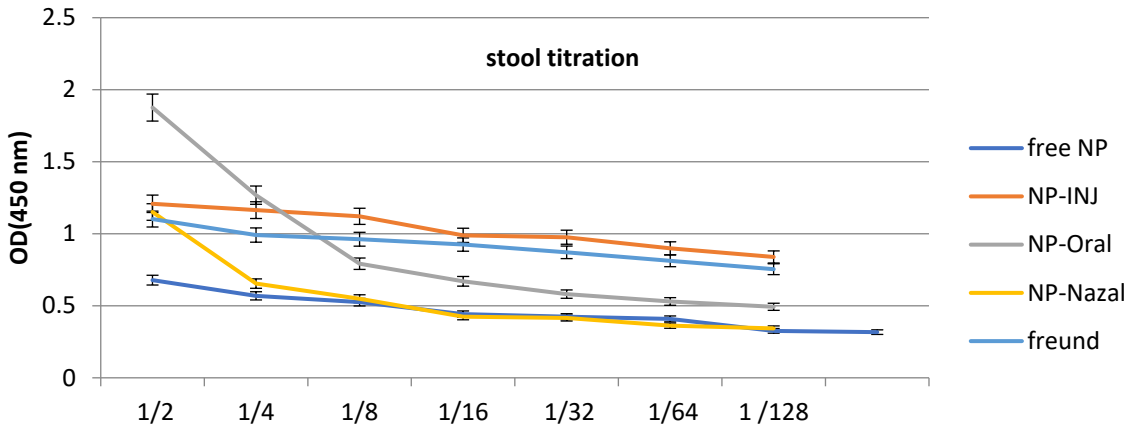

Figure 5. Stool titration diagram

Bugybayeva et al.,[18] conducted a study in 2021 to test a new candidate vaccine for human malaria based on influenza virus vectors using a guinea pig model. The study aimed to evaluate the efficacy of the newly developed brucellosis vaccine by examining its protective effect after conjunctival, intranasal, and sublingual administration at doses of 105 EID50, 106 EID50, and 107 EID50, respectively, and comparing it with B. melitensis viral strain 16 M and the commercial vaccine B. melitensis Rev 1. The study observed a gradual increase in body weight among guinea pigs after primary and booster vaccination with the vaccine, using conjunctival, intranasal, and sublingual injection routes and different vaccine doses. The most successful way to use the created vaccine was to immunize guinea pigs twice intranasally with a 106EID50 dosage, which provided 80% protection against B. melitensis 16 M infection ($P<0.05$), comparable to the efficacy of the commercial B. melitensis Rev.1 vaccine. These promising results indicate the potential for the proposed vaccine to provide effective protection, warranting further promotion and development.[18]

The researchers at Paolino et al.[19] wanted to look into the possibility of bola-surfactant niosomes as local delivery systems for 5-fluorouracil (5-FU) in the treatment of skin cancer. As a topical administration approach, a novel niosome system composed of hexadecyl-bis-(1-aza-18-crown-6) (Bola), Spanner 80®, and cholesterol (molar ratio 2:5:2) was presented. The average size of the Bola-niosomes was approximately 400 nm, which was decreased to roughly 200 nm by a polyethylene test method after reaching 0.1%. Furthermore, the Bola-niosomes exhibited a loading capacity of approximately 40% based on the added amount of 5-FU during preparation. The 5-FU-loaded Bola-niosomes were examined for cytotoxicity on Skmel-28 (human melanoma) and Hacat (non-melanoma skin cancer with particular mutations in the P53 tumor suppressor gene). The study measured the effectiveness of the drug-carrying niosomes in comparison to free drugs. The results demonstrated that 5-FU-loaded Bola-niosomes showed an improved cytotoxic effect compared to the drug alone. Furthermore, bola-niosomes increased drug penetration by eight and four times, respectively, when compared to an aqueous drug solution and a mixture of empty bola-niosomes and an aqueous drug solution. Overall, the findings of this study showed promising results for the potential application of bola-surfactant niosomes as a local delivery system for the treatment of skin cancer with 5-FU.[19]

Barani et al.[20] conducted a study to investigate the efficacy of niosomes loaded with lawsone for their antitumor activity in the MCF-7 breast cancer cell line. The study found that the low solubility of phytochemical compounds in aqueous environments can cause low bioavailability and permeability, as well as a lack of stability in biological environments. To address this issue, niosomes were prepared using the thin film hydration (TFH) method and then loaded with henna extract (HLaw) and standard Lawsone (SLaw). The two resulting formulations were

compared, and experiments were conducted to evaluate the antitumor activity in the MCF-7 cell line. Overall, the study found that niosomes containing lawsone, specifically SLaw, had considerable anticancer activity in the MCF-7 cell line. The results highlight the potential for niosomes to be utilized as a promising delivery system for phytochemical compounds in cancer treatment. The study revealed that the niosomes had distinct spherical shapes with a particle size of approximately 250 nm in diameter, and negative zeta potentials. Moreover, the niosomes were stable for up to two months at 4°C. Both formulations exhibited an entrapment efficiency of around 70% and maintained a sustained release profile. In vitro studies revealed that the niosomes utilized for encapsulation increased anticancer activity in the MCF-7 cell line significantly more than Law's solution alone. The findings suggest that using niosomes as a delivery mechanism for phytochemical substances with low solubility in bodily fluids is a promising approach.[20] Overall, this study highlights the potential of using niosomes as a carrier system to improve the delivery and efficacy of phytochemical compounds with low solubility, particularly in cancer treatment.[20]

Conclusions

Two promising technologies of the 21st century is biotechnology and nanobiotechnology, which involve the design, development, and application of materials and devices at the nanoscale. Because of their unique capacity to encapsulate both hydrophilic and hydrophobic medicines, niosomes, a form of vesicle frequently utilized in drug delivery systems, are attracting increased interest, notably in vaccine development. Our study has successfully demonstrated that niosomes are a more effective and safer option for Brucella vaccine development. The results suggest that the use of niosomal formulations can offer improved efficacy and safety compared to traditional vaccine formulations. These findings have significant implications for the development of more efficient and effective vaccines for a variety of diseases, boosting prospects for better healthcare outcomes.

Acknowledgment

None.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

World Health Organization: WHO; Bovine serum albumin: BSA; Thin film hydration: TFH; Standard Lawsone: Slaw.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of Baqiyatullah University of Medical Sciences, Tehran, Iran (code: IR.BMSU.REC.1397.373).

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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