Medical and Pharmaceutical Journal

Volume 2 Issue 3

Med. Pharm. J. Original article

Production and Characterization of Recombinant Multi-Epitope G Glycoprotein of Rabies Virus

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DOI: 10.55940/medphar202351
Submitted: 07- Sep-2023
Accepted: 25- Sep -2023
Published:10-Oct-2023

Abstract

Background: Therapeutic proteins have gained increasing interest in research and development for treatments and vaccines against numerous diseases in recent years. This study focuses on producing a recombinant multi-epitope protein of the G glycoprotein, which will serve as a novel vaccine candidate against rabies virus.

Aim: This research aimed to consolidate various 'neutralizing' B epitopes into a single protein and investigate its biochemical and biological characteristics.

Material and Method: The recombinant multi-epitope G glycoprotein was synthesized after transforming the E. coli BL21 bacterial strain with the genetic construct. The protein was produced in soluble form following bacterial induction with 1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside) and purified using a Ni-NTA affinity column. The 'multi-epitope' protein was characterized by 12% SDS-PAGE. The specificity of binding of the novel recombinant G glycoprotein to human (serum) rabies antibodies was compared to the binding of these same antibodies to whole and inactivated rabies viruses available in the laboratory, namely, the rabies PV-11 strain and the CVS-11 strain, using an indirect ELISA test.

Result: The results demonstrate that the E. coli BL21 strain successfully produced the new recombinant protein with a molecular weight of 30 kDa and that the loss of glycosylation has no effect on the immunogenicity of this protein. Structurally, the new recombinant protein has preserved the epitopes recognized by serum from hyperimmune individuals.

Conclusion: These findings suggest the potential efficacy of the multi-epitope recombinant G glycoprotein as a vaccine candidate and the possibility of developing a novel vaccine based on this multivalent recombinant protein.

Keywords: Epitopes, G Glycoprotein, Immunization, Rabies, Vaccine.

INTRODUCTION

Rabies, a zoonotic viral disease caused by the rabies virus (RABV), remains a significant public health concern worldwide. Rabies kills approximately 59,000 people -worldwide each year. In developed countries, such as the United States, dog vaccines make rabies less common. Dogs that have not been vaccinated are a common source of rabies transmission, particularly in underdeveloped countries where access to the vaccine may be limited [1].

Rabies virus is most transmitted to its host through the bite of an infected animal. Initially, it multiplies in muscle cells at the site of inoculation. Then, it targets the neurons, which are the most susceptible to its effects. Unlike most other viruses, the spread of the rabies virus does not occur through the bloodstream; instead, it follows a neural pathway. The virus enters the nervous system, traveling to the brain, where it replicates. It then returns to the periphery and nerve endings through the peripheral nervous system, eventually reaching specific organs such as the heart, eyes, or kidneys. Of utmost importance is the involvement of the salivary glands, where viral replication is intense, facilitating the transmission of the virus by an infected animal during a bite [2]. Despite the availability of effective vaccines for post-exposure prophylaxis, rabies still claim tens of thousands of lives each year, particularly in regions with limited access to healthcare resources. The development of novel and improved rabies vaccines is thus a critical priority in the global efforts to control and eliminate this deadly disease [3,4].

In recent years, vaccinology has witnessed substantial advancements, particularly in designing and producing recombinant proteins with enhanced immunogenicity [5]. Such therapeutic proteins have emerged as valuable tools for developing vaccines for infectious, inflammatory, autoimmune, and cancer-related diseases. These recombinant proteins offer several advantages, including their potential for targeted antigen presentation, cost-effective production, and reduced safety concerns compared to traditional approaches involving live or inactivated pathogens [6,7].

This research article looks into producing and characterizing a recombinant multi-epitope G glycoprotein of the rabies virus (RABV). This study aimed to consolidate various neutralizing B epitopes from the rabies virus into a single protein construct, thereby creating a novel vaccine candidate against rabies. The authors explore the structural and immunogenic properties of this multi-epitope G recombinant protein through a combination of genetic engineering, bacterial expression, and biochemical analyses.

MATERIAL AND METHODS

1. Production of Recombinant Multi-Epitope Glycoprotein G by E. coli BL21

An overnight pre-culture of the bacterial strain E. coli BL21 was inoculated in 100 ml of Luria-Bertoni (LB) + Kanamycin (75 µg/ml) and incubated at 37°C with agitation (250 rpm) for three hours until reaching the exponential growth phase. Production of the recombinant multi-epitope glycoprotein G was induced by adding 100 µl of IPTG (1 M). Induction was continued under the same incubation conditions for five hours.

2 **Extraction of Recombinant Multi-Epitope Glycoprotein G**

Following induction, BL21 bacteria were harvested by centrifugation at 4000 rpm for 20 minutes at 4°C. The bacterial pellet was resuspended in 3 ml of bacterial lysis buffer and subjected to five cycles of sonication on ice (Amplitude: 40%, pulse: 15 sec, rest: 10 sec). The sonication product was then centrifuged at 13000 rpm for 20 minutes at 4°C, and the supernatant containing soluble proteins was collected for the purification step.

3. **Purification of Recombinant Multi-Epitope Glycoprotein G by Affinity Chromatography on Ni-NTA column**

The recombinant multi-epitope glycoprotein G was purified using affinity chromatography on the Ni-NTA column (Qiagen, France). This column contained resin beads complexed with Ni2+ ions that have a strong affinity for the imidazole core of the histidine tag (His 6x) located at the C-terminal end of this recombinant protein.

A column consisting of 500 µl of Ni-NTA coupled to Sepharose beads was washed with 5 ml of PBS (1x, pH 7.4) and then equilibrated with 10 ml of PBS (1x, pH 7.4). Once the column was ready, the supernatant was passed through the column ten times. Subsequently, a wash with PBS (1x, pH 7.4) was performed, followed by a second wash with (phosphate-buffered saline) PBS (1x, pH 7.4) containing 20 mM Imidazole to elute nonspecifically bound proteins. Elution with a high concentration of 250 mM Imidazole allowed the release of the recombinant multi-epitope glycoprotein G, which competed with the imidazole cores present on the histidine tag (His 6x).

4. Measurement of Recombinant Multi-Epitope Glycoprotein G

The quantity of the recombinant multi-epitope glycoprotein G after purification was determined using a colorimetric assay following the Bicinchoninic Acid Assay (BCA) method [8], using a standard range of a bovine serum albumin (BSA) solution (2 mg/ml). This method is based on the reduction of Cu2+ ions to Cu+ ions: Cu2+ ions in the alkaline BCA reagent react with the peptide chains of proteins, and the formed Cu+ cations utilize bicinchoninic acid to form a colored complex (navy blue) that absorbs at a wavelength of 540 nm.

5. **Characterization of Recombinant Multi-Epitope Glycoprotein G by 12% Polyacrylamide Gel Electrophoresis**

The purified recombinant multi-epitope glycoprotein G was analyzed by 12% polyacrylamide gel electrophoresis in the presence of Sodium Dodécyl Sulfate (SDS). This gel consisted of a stacking gel (4%) to consolidate proteins at the same migration level and a separating gel (12%) to fractionate different proteins based on their molecular weights. A volume of 20 µl per sample was loaded into the gel wells. Electrophoretic migration was performed at 100 V for one hour at room ambient temperature. Each migration was standardized using a protein marker containing proteins of known sizes and concentrations. After migration, the gels were stained in a staining buffer under constant slow agitation for at least 1 hour and then destained in a destaining buffer.

6. Characterization of the Specificity of Recombinant Multi-Epitope Glycoprotein G by Indirect ELISA

The purified recombinant multi-epitope glycoprotein G was assessed using an indirect ELISA [9] by its recognition of human rabies antibodies. The binding

specificity of the recombinant protein to human rabies antibodies was compared to laboratory-available viruses: the inactivated rabies strains CVS-11 and PV-11. For this purpose, an ELISA plate (Maxisorp, NUNC, Denmark) was coated with 100 µl per well of inactivated CVS-11 and PV-11 strains (0.1 IU per well) and the recombinant multi-epitope glycoprotein G (10 µg/ml) in PBS buffer (1x, pH 7.4) and left to incubate for 16 hours at 4°C. The following day, the plate was washed once with PBS (1x, pH 7.4) to remove unbound proteins and viruses and then blocked with 100 µl of PBS-BSA2% per well for one hour at room ambient temperature. After another wash with PBS (1x, pH 7.4), (100 µl per well) of human rabies serum samples diluted 1/100 in PBS-BSA2% were added to the plate and incubated for 2 hours. The plate was then washed five times with PBS-Tween 0.1% and five times with PBS (1x, pH 7.4). Bound antibodies were detected by adding 100 µl per well of secondary goat anti-human IgG antibodies conjugated to peroxidase, diluted 1/2000 in PBS-BSA2%, for one hour at room ambient temperature. Unbound antibodies were removed after five washes with PBS- between 0.1% and five washes with PBS (1x, pH 7.4). Revelation was achieved with 100 µl per well of 3,3', 5,5'Tétraméthyl benzidine (TMB) substrate, and after a 20-minute incubation in the dark, the revelation reaction was stopped by adding 50 µl of 2N sulfuric acid (H2SO4) to each well. Absorbance was measured at 450 nm using an ELISA reader.

7. Titration of Human Rabies Serum

Two ELISA plates (Maxisorp, NUNC, Denmark) were coated with 100 µl per well of the recombinant multi-epitope glycoprotein G (10 µg/ml) in PBS buffer (1x, pH 7.4) and left to incubate for 16 hours at 4°C. The next day, the plates were washed once with PBS (1x, pH 7.4) to remove unbound proteins and blocked with 100 µl of PBS-BSA2% per well for one hour at room ambient temperature. After another wash with PBS (1x, pH 7.4), 100 µl per well of human rabies serum samples diluted in PBS-BSA2% were added to the plates. The dilutions used were 1/10, 1/20, 1/100, 1/200, 1/400, 1/800, 1/1000, and 1/5000, respectively. These two plates were incubated for 2 hours and then washed five times with PBS-Tween 0.1% and five times with PBS (1x, pH 7.4). Bound antibodies were detected by adding 100 µl per well of secondary goat anti-human IgG antibodies conjugated to peroxidase and diluted 1/2000 in PBS-BSA2% for one hour at room temperature. Unbound antibodies were removed after five washes with PBS-Tween 0.1% and five washes with PBS-Tween 0.1%. Revelation was performed using 100 µl per well of TMB substrate, and after a 20-minute incubation in the dark, the revelation reaction was stopped by adding 50 µl of 2N sulfuric acid (H2SO4) to each well. Absorbance was read at 450 nm using an ELISA reader.

8 Competition of Binding of Human Rabies Antibodies to CVS-11 Virus by Soluble Recombinant Multi-Epitope Glycoprotein G

Two ELISA plates (Maxisorp, NUNC, Denmark) were coated with 100 µl per well of inactivated Challenge Virus Standard (CVS)-11 rabies strain (0.1 IU per well) in PBS buffer (1x, pH 7.4) and left to incubate for 16 hours at 4°C. The next day, the plates were washed once with PBS (1x, pH 7.4) to remove unbound viruses and then blocked with 100 µl PBS-BSA 2% per well for one hour at room temperature. After

another wash with PBS (1x, pH 7.4), the first plate received (100 µl per well) human rabies serum diluted in PBS-BSA2%, while the second plate received (100 µl per well) human rabies serum diluted in PBS-BSA2% mixed with the recombinant multi-epitope glycoprotein G. These two plates were incubated for 2 hours and then washed five times with PBS-Tween0.1% and five times with PBS (1x, pH 7.4). Bound antibodies were detected by adding 100 µl per well of secondary goat anti-human IgG antibodies conjugated to peroxidase and diluted 1/2000 in PBS-BSA2% for one hour at room ambient temperature. Unbound antibodies were removed after five washes with PBS-between 0.1% and five washes with PBS (1x, pH 7.4). Revelation was achieved with 100 µl per well of TMB substrate, and after a 20-minute incubation in the dark, the revelation reaction was stopped by adding 50 µl of 2N sulfuric acid (H2SO4) to each well. Absorbance was read at 450 nm using an ELISA reader.

9 Preparation of Mice for Immunization and Immunization Protocol

Mice (BALB/c) were used to study the immunogenicity of the recombinant multi-epitope glycoprotein G. For this, 15 non-pregnant female BALB/c mice aged between six to eight weeks were divided into 3 cages:

- Cage 1: Contained 5 female BALB/c mice injected subcutaneously with (50 µg/mouse) of the recombinant multi-epitope glycoprotein G.
-Cage 2: Contained 5 female BALB/c mice injected intraperitoneally with (50 µg/mouse) of the recombinant multi-epitope glycoprotein G.
-Cage 3: Contained 5 female BALB/c control mice that did not undergo any injection.

The immunization protocol involved injecting the BALB/c mice with the recombinant multi-epitope glycoprotein G mixed with (ACF) either intraperitoneally or subcutaneously on days D0. On days D14 and D28, the same mice are injected with the recombinant multi-epitope glycoprotein G mixed with (AIF) either intraperitoneally or subcutaneously.

10 Collection and Analysis of Blood from Immunized Mice

Seven days after the last immunization, blood samples were collected from the retro-orbital sinus. Blood extracts were centrifuged for 15 minutes at 4°C at 13000 rpm to recover serums. The recovered serums underwent decomplementation for 30 minutes in a water bath at a temperature of 56°C. This temperature ensured the destruction of complement C1q, the presence of which could affect the ELISA signal.

11 Evaluation of Humoral Immunity Conferred by Immunization

Decomplemented serums were tested by indirect ELISA for their binding capacity to the rabies virus: the PV-11 strain or the CVS-11 strain. To do this, the ELISA plate (Maxisorp, NUNC, Denmark) was coated with 100 µl of the rabies virus: the PV-11 strain, inactivated CVS-11 rabies strain (0.1 IU per well) in PBS buffer (1x, pH 7.4) for 16 hours at 4°C. The next day, the plate was washed once with PBS (1x, pH 7.4) to remove unbound viruses and blocked with 100 µl PBS-BSA2% per well for one hour at room temperature. After another wash with PBS (1x, pH 7.4), 100 µl per well of decomplemented mouse sera diluted 1/100 in PBS-BSA2% was added. The plate was incubated for 2 hours and then washed five times with PBS-Tween 0.1% and five times with PBS (1x, pH 7.4). Bound antibodies were detected by adding 100 µl

per well of secondary goat anti-mouse IgG antibodies conjugated to peroxidase and diluted 1/2000 in PBS-BSA 2% for one hour at room temperature. Unbound antibodies were removed after five washes with PBS-Tween 0.1% and five washes with PBS (1x, pH 7.4). Revelation was performed with 100 µl per well of TMB substrate after incubation for 20 minutes in darkness; the revelation reaction was terminated by adding 50 µl of 2N sulfuric acid (H2SO4) to each well. The absorbance was measured at 450 nm using an ELISA reader.

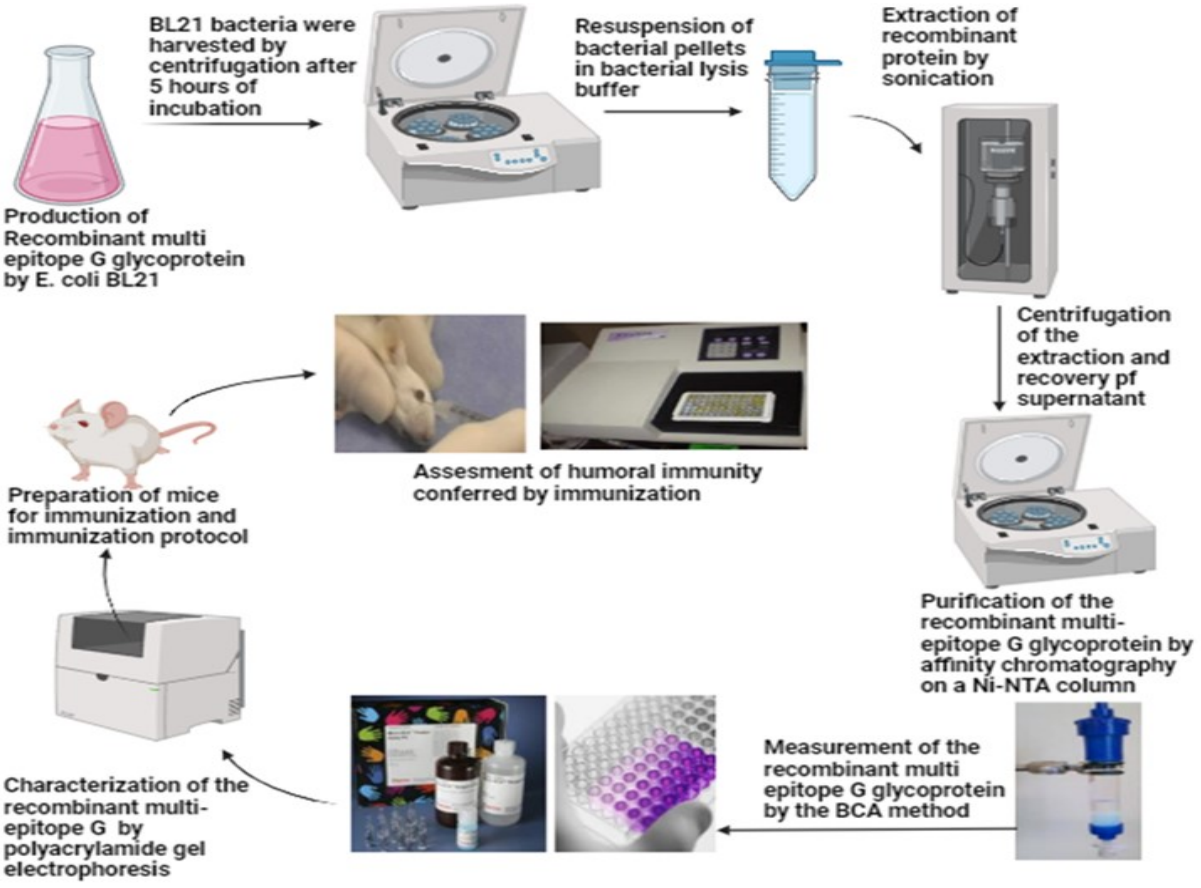

Figure 1: The stages involved in the production and characterization of the Recombinant Multi-Epitope G Glycoprotein derived from the Rabies Virus

RESULTS

1 Production of Recombinant Multi-Epitope Glycoprotein G by E. coli BL21

Recombinant multi-epitope glycoprotein G was synthesized using an E. coli strain containing a plasmid composed of a Kanamycin resistance gene and a promoter. A pre-culture was prepared, and production was induced by adding IPTG, which is considered an analog of allolactose. Unlike allolactose, IPTG is not degraded by β-galactosidase and thus remains at a constant concentration, allowing continuous production of

recombinant multi-epitope glycoprotein G. Induction continued under the same incubation conditions for a duration of five hours. To extract the protein of interest from the culture medium, it was centrifuged for 20 minutes at 4000 rpm at 4°C.

2. Purification of Recombinant Multi-Epitope Glycoprotein G by Ni-NTA Column Chromatography

The BL21 (DE3) bacteria producing recombinant multi-epitope glycoprotein G were lysed by sonication, and bacterial extracts were purified using Ni-NTA chromatography. The first wash with PBS (1x, pH 7.4) removed unbound bacterial proteins, and the second wash with PBS (1x, pH 7.4) contained a low concentration of 20 mM Imidazole detached bacterial proteins that were not specifically bound to the column. Protein elution was achieved using a high concentration of 250 mM Imidazole. The concentration of purified recombinant multi-epitope glycoprotein G was approximately 1 mg/ml, estimated using the BCA method.

3. Analysis of Recombinant Multi-Epitope Glycoprotein G by 12% SDS-PAGE

The recombinant multi-epitope glycoprotein G was analyzed on a 12% polyacrylamide gel in the presence of SDS. A band of approximately 30 kDa, corresponding to the estimated size of the new recombinant multi-epitope protein, was observed (Figure 2).

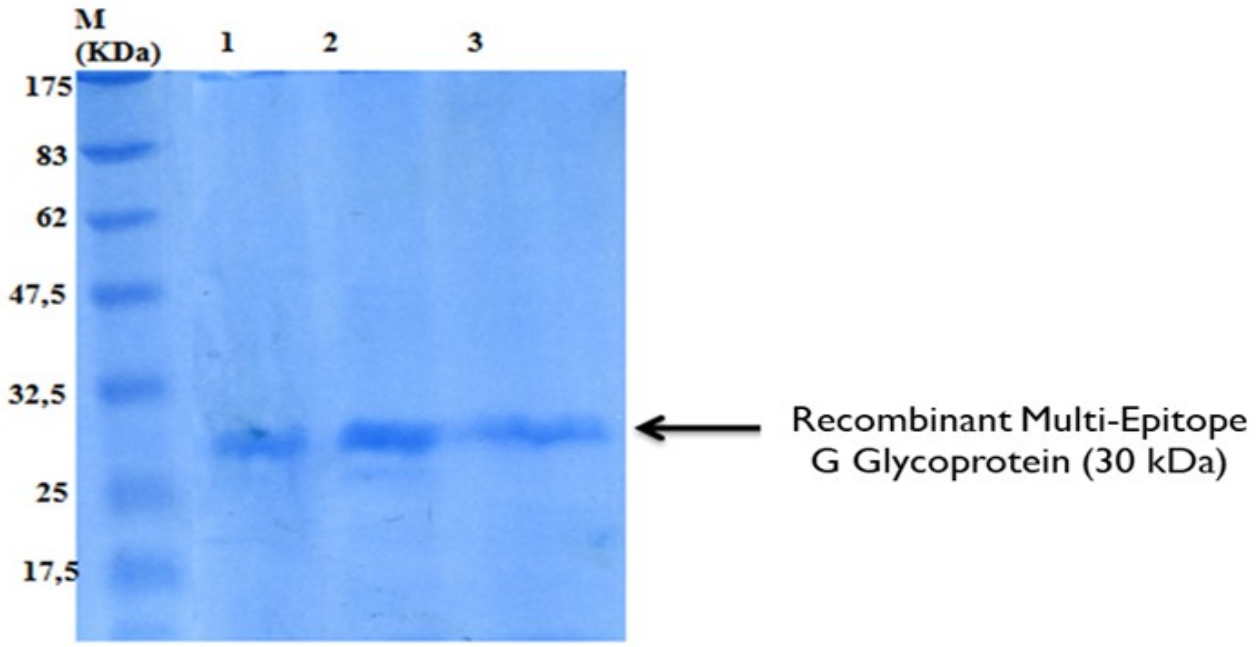

Figure 2: SDS-PAGE 12% electrophoretic profile of recombinant multi-epitope glycoprotein G, stained with Coomassie blue. The recombinant multi-epitope glycoprotein G, extracted by sonication and purified by affinity chromatography, was analyzed on a 12% polyacrylamide gel in the presence of SDS. M: molecular weight marker. Wells 1 to 3: first, second, and third elutions with PBS-Imidazole 250 mM.

4. Determination of the Specificity of Recombinant Multi-Epitope Glycoprotein G by Indirect ELISA

The specificity of binding of recombinant multi-epitope glycoprotein G to human rabies antibody was investigated and compared to laboratory-available viruses: PV-11 strain and inactivated CVS-11 strain. The results obtained indicate that human rabies antibodies bind to the PV-11 virus with absorbance values ranging from 0.20 to 0.44 AU. In contrast, in the case of the CVS-11 virus, the values are higher than those for PV-11, ranging from 0.52 to 0.78 AU. In the case of recombinant multi-epitope glycoprotein G, human rabies antibodies specifically bound to it with absorbance values greater than 0.80 AU. These results demonstrate that recombinant multi-epitope glycoprotein G has retained the epitopes recognized by human serum from individuals vaccinated against rabies (Figure 3). The ELISA plate was coated with recombinant multi-epitope glycoprotein G (10 µg/ml) and rabies virus; PV-11 strain, inactivated CVS-11 strain (0.1 IU/well) (100 µl/well). After saturation, diluted human rabies sera (1/100) in PBS-BSA2% were added and incubated with PV-11, CVS-11, and recombinant multi-epitope glycoprotein G. They were detected by secondary goat anti-human IgG antibodies conjugated to peroxidase (diluted 1/2000). Revelation was performed by adding the TMB substrate, and absorbances were measured at 450 nm using an ELISA reader.

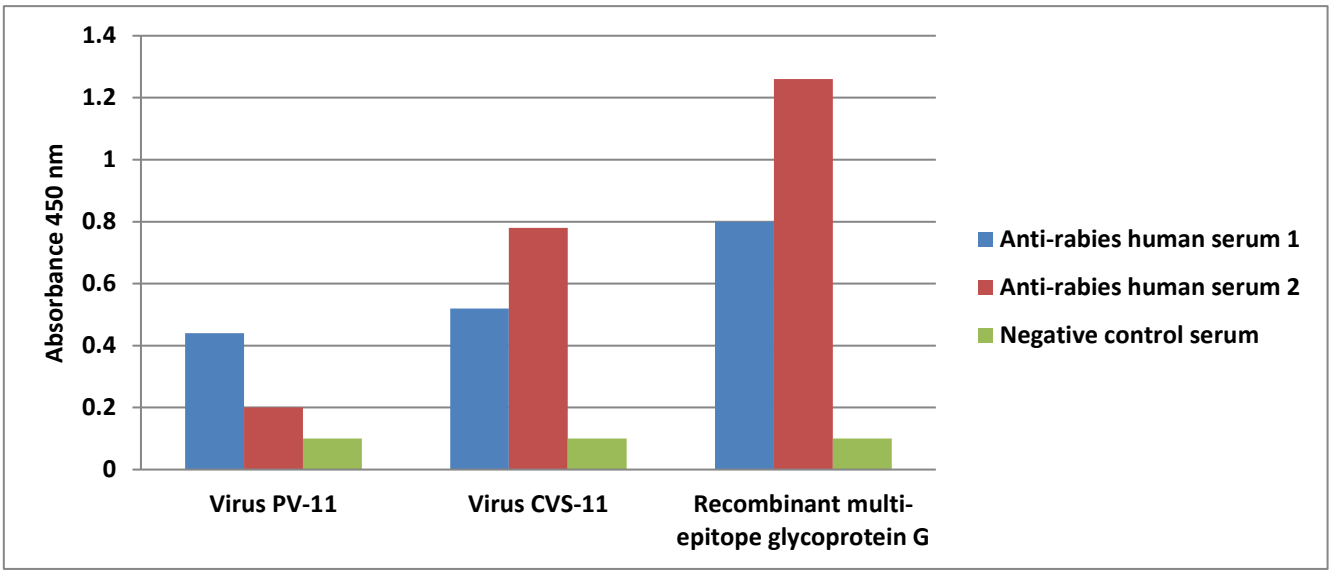

Figure 3: Characterization by Indirect ELISA of the Recognition of Recombinant Multi-Epitope Glycoprotein G by Human Rabies Antibodies.

5. Titration of Human Rabies Serum

This procedure demonstrates that the polyclonal antibodies present in human rabies serum recognize recombinant multi-epitope glycoprotein G adsorbed on a solid support. The titration of human rabies serum shows a dose-response effect and a correlation between the concentration of human rabies antibodies and the absorbance value observed from the 1/100 dilution. The recognition of recombinant

multi-epitope glycoprotein G is maintained at a high dilution of rabies serum (Figure 4). After saturation, diluted human rabies serum (1/10, 1/20, 1/100, 1/200, 1/400, 1/800, 1/1000, and 1/5000) in PBS-BSA2% was added to both plates. Then, secondary goat anti-human IgG antibodies conjugated to peroxidase (diluted 1/2000) were added, and the revelation was carried out using TMB. Absorbances were measured at 450 nm using an ELISA reader.

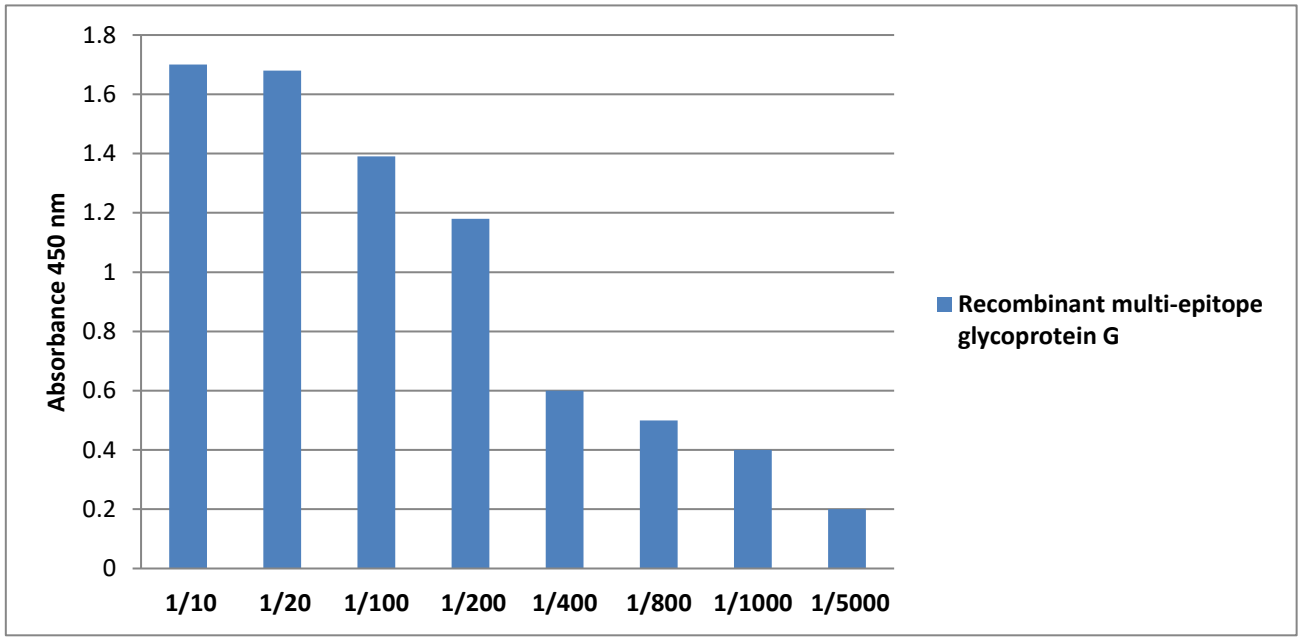

Figure 4: Characterization of Human Rabies Serum. Two ELISA plates were simultaneously coated with recombinant multi-epitope glycoprotein G (100 µl/well).

6 Competition Binding of Rabies Antibodies to CVS-11 Virus by Recombinant Multi-Epitope Glycoprotein G in Soluble Form

By conducting competition tests between the soluble protein and glycoprotein G expressed on the surface of rabies virus (natural trimer), it was observed that human rabies antibodies mixed with recombinant multi-epitope glycoprotein G bound to inactivated CVS-11 strain with absorbance values lower than those obtained with rabies antibodies alone (without recombinant multi-epitope glycoprotein G). The results obtained show that the prior incubation of polyclonal antibodies with the recombinant G protein induces competition for binding to the whole rabies virus adsorbed at the bottom of the wells of an ELISA plate (Figure 5) Two ELISA plates were simultaneously coated with inactivated rabies virus strain (CVS-11) (100 µl/well). After saturation, diluted human rabies serum (1/100) in PBS-BSA2% was added and incubated with the virus in the first plate, while in the second plate, diluted human rabies serum (1/100) in PBS-BSA2% mixed with recombinant multi-epitope glycoprotein G was added and incubated with the virus. Then, secondary goat anti-human IgG antibodies conjugated to peroxidase (diluted 1/2000) were added. Revelation was performed by adding the TMB substrate, and absorbances were measured

at 450 nm using an ELISA reader.

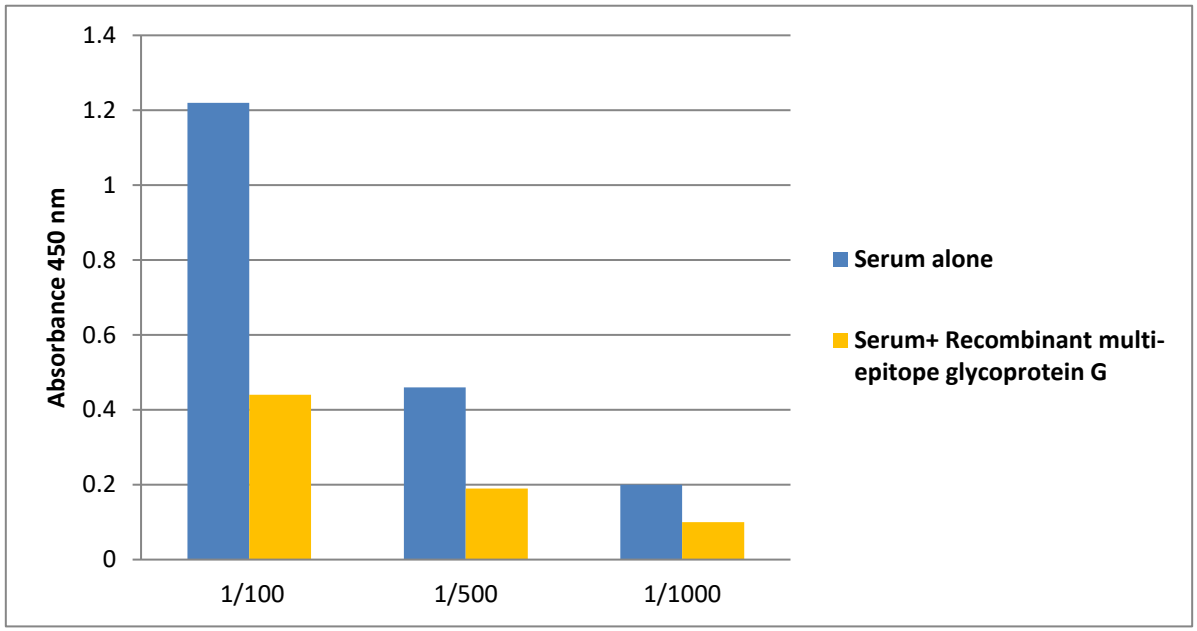

Figure 5: Study of the competition binding of human rabies antibodies to CVS-11 virus by soluble recombinant multi-epitope glycoprotein G.

7. Immune Response Induced in BALB/c Mice Immunized with Recombinant Multi-Epitope Glycoprotein G

The effect of recombinant multi-epitope glycoprotein G in inducing a humoral response in BALB/c mice was studied. These female BALB/c mice (5 mice/group) were inoculated by two different routes: subcutaneous and intraperitoneal, with a dose of 50 µg of recombinant multi-epitope glycoprotein G mixed with ACF during the first injection and 50 µg of recombinant multi-epitope glycoprotein G mixed with AIF during the second and third injections. Mice immunized with this recombinant protein were followed for 30 days and compared to a negative control group consisting of non-immunized mice. We collected the following serums: serums from mice immunized by subcutaneous and intraperitoneal routes and serums from non-immunized mice. We examined the recognition of the rabies virus by murine antibodies induced by the recombinant multi-epitope glycoprotein G in an indirect ELISA test. The aim of this test was to assess the immunogenicity of the recombinant multi-epitope glycoprotein G in BALB/c mice. The obtained serums were tested to detect the rabies virus. Figure 6 shows that the signals from serums obtained from non-immunized mice were weak and not significant (0.25 to 0.29 AU units), while the signals from serums of immunized mice were substantial (2.37 to 3.25 AU units). This demonstrates that the recombinant multi-epitope glycoprotein G has a high capacity to induce a humoral response directed against the rabies virus. It was also observed that the highest ELISA signals were obtained in mice immunized intraperitoneally. These results show that the recombinant multi-epitope

glycoprotein G can induce a humoral response directed against the whole rabies virus, regardless of the mode of administration (Figure 6).

The ELISA plate was coated with rabies virus; the PV-11 strain, inactivated CVS-11 strain (0.1 IU/well); (100 µl/well). After saturation, murine sera, depleted of complement and diluted (1/100) in PBS-BSA2%, were added and incubated with PV-11 and CVS-11. Murine anti-rabies antibodies were detected using secondary goat anti-mouse IgG antibodies conjugated with peroxidase (diluted 1/2000). Revelation was achieved by adding the TMB substrate, and absorbances were measured at 450 nm using an ELISA reader.

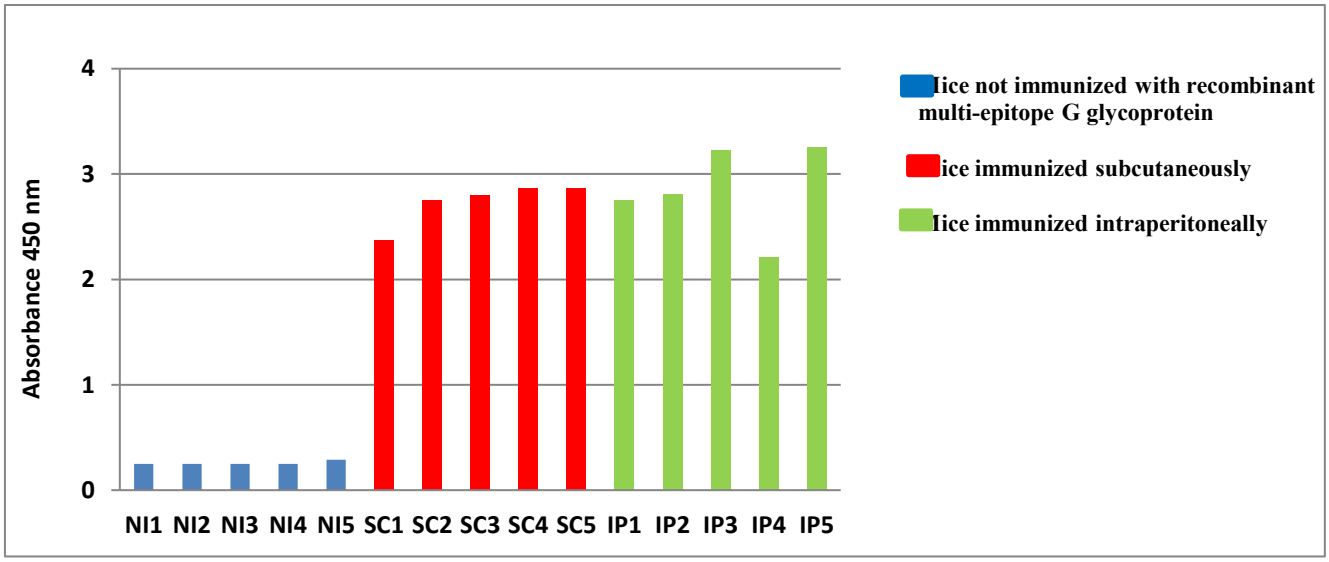

Figure 6: Study of the immunogenicity of the recombinant multi-epitope glycoprotein G administered to BALB/c mice via subcutaneous (SC) or intraperitoneal (IP) routes

DISCUSSION

Recombinant proteins are of increasing interest in the research and development of therapeutic molecules and vaccines for various infectious diseases [11], inflammatory diseases [12], autoimmune diseases, and cancer [13]. The applications of these biomolecules extend beyond therapy and diversify into diagnostics.

In this study, we produced a recombinant multi-epitope protein of the G glycoprotein, which will serve as a novel vaccine candidate against rabies virus. To achieve this, we grouped neutralizing B epitopes into a single genetic construct, which was inserted into the E. coli strain BL21 through chemical transformation. Several experimental approaches were employed to improve the production of the recombinant multi-epitope G glycoprotein. First bacterial culture conditions were altered, testing different volumes of Luria-Bertoni (LB) (ranging from 100 ml to 200 ml) and various bacterial growth temperatures. The soluble recombinant protein, characterized by SDS-PAGE, exhibited a molecular weight

of 30 kDa. The specificity of recognition of the novel recombinant multi-epitope G glycoprotein by human anti-rabies serum antibodies was confirmed through an indirect ELISA test. It was compared to the binding of these same polyclonal antibodies to whole, inactivated rabies viruses available in the laboratory, namely the rabies PV-11 strain and the CVS-11 strain. Human anti-rabies antibodies specifically bound to the recombinant multi-epitope glycoprotein with absorbance values higher than those obtained with the PV-11 and CVS-11 strains, indicating that structurally, the novel recombinant multi-epitope protein produced by E. coli retained the B epitopes recognized by serums from individuals hyperimmunized against rabies. Furthermore, through competitive binding tests between soluble protein and G glycoprotein expressed on the surface of the rabies virus (natural trimer), pre-incubation of polyclonal antibodies with the recombinant protein induced a reduction (or inhibition) in their binding to the whole rabies virus adsorbed at the bottom of an ELISA plate well. The inhibition of binding of human polyclonal antibodies to CVS-11 viruses could be attributed to saturation of the paratopes of polyclonal antibodies, better access of antibodies to the recombinant protein than to the spike, and the quantity of the recombinant multi-epitope G glycoprotein being higher than that of the virus coating.

The study of the immunogenic properties of the recombinant multi-epitope protein was determined in vivo in BALB/c mice. The recombinant multi-epitope G glycoprotein (50 µg/mouse), mixed with ACF, was injected on day 0 into two groups of mice intraperitoneally to promote macrophage activation, leading to better antigen presentation or subcutaneously to activate Langerhans cells. On days 14 and 28, the mice received an injection of the recombinant multi-epitope G glycoprotein mixed with AIF. Injecting the protein combined with ACF induces a mixed humoral and cellular response. The humoral component of the induced response is linked to the oil-in-water emulsion, while the cellular component is attributable to mycobacterial extracts [14]. ACF allows for a prolonged presence of the recombinant multi-epitope G glycoprotein, which activates macrophages further. These macrophages break it down and present it, in association with MHC II molecules, to specific immune cells. The two subsequent injections of the protein mixed with AIF help boost the humoral response. This immunogenicity was demonstrated by the induction of a humoral response based on the production of neutralizing IgG antibodies recognized by the rabies virus (CVS-11 and PV-11) in BALB/c mice injected with this protein. The obtained serums were tested to detect the rabies virus through an indirect ELISA test, and it was observed that the recombinant multi-epitope G glycoprotein could induce

a humoral response directed against the entire rabies virus, regardless of the mode of administration.

In this study, the quantity of adjuvants administered to mice was limited due to their harmful side effects on their health. Indeed, repeated injections of the recombinant multi-epitope G glycoprotein in the presence of an adjuvant (ACF) can lead to fatal anaphylactic reactions. For these reasons, ACF was administered to the mice only during the first injection, and to inject AIF only during the booster injections.

To validate the use of this new protein as a rabies vaccine molecule, it is essential to immunize a large number of BALB/c mice with different quantities of proteins, followed by injections (challenge) of increasing doses of virulent rabies virus via intramuscular or intracerebral routes. The survival of mice at high lethal doses after this 'challenge' test will indicate the immunogenic and protective power of the 'multi-epitope' protein and thus its application as a 'vaccine' molecule.

The use of the new recombinant protein for rabies vaccination offers several advantages. It avoids contact with virulent rabies viruses and thus allows work under normal conditions without the risk of rabies virus infection. Indeed, obtaining this protein does not require specialized viral inactivation laboratories that must provide elevated levels of safety (P2 and P3 levels). Furthermore, the production of the recombinant G glycoprotein is simple and does not require extensive equipment such as hoods and highly selective air filtration systems. It also eliminates various steps in cell culture, such as inactivating virulent viruses with beta-propiolactone. Additionally, the production method of the recombinant multi-epitope G glycoprotein used in the laboratory constitutes an interesting alternative to the conventionally used protocol (eukaryotic pathway), which offers not only significant yield but also time and cost savings. The prokaryotic system is a straightforward system as it merely involves introducing the gene to be expressed into one of the numerous available plasmid vectors for bacterial transformation. This system is highly amenable to large-scale fermenter culture and can yield large quantities of proteins (up to several grams per liter). Production is rapid because the bacterial doubling time is around half an hour. Finally, it is cost-effective due to the simplicity of mass culture. Indeed, prokaryotic expression promotes overproduction and purification of a recombinant protein, often faster, cheaper, and easier than in other organisms.

CONCLUSION:

In conclusion, while current rabies vaccines are produced using inactivated viruses, this study represents a significant advancement by developing a rabies vaccine based on a recombinant multi-epitope protein derived from the G

glycoprotein. The experimental results clearly demonstrate the vaccine's efficacy in protecting against rabies in mice. The potential for further application of this vaccine in the future, particularly in the immunization of dogs in underdeveloped regions, holds promise for reducing the incidence of rabies-related fatalities in humans.

ACKNOWLEDGEMENTS

Ethical approval: Our research was done under ethical approval received from the department of Microbiology, Pasteur Institute of Tunis.

Conflect of interest

The authors declare no conflict of interest.

Authors' contributions: All authors made contributions through the designing, drafting, and analysis of the research work. We all have mutual interest, no conflicts of interest. This work has not been published elsewhere.

Funding

None

Abbreviation:

BCA: Bicinchoninic Acid Assay

BSA: bovine serum albumin

CVS: Challenge Virus Standard

kDa : Kilodalton

LB : Luria-Bertoni

Ni-NTA: Nickel-Nitrilo Triacetic Acid

PBS : Phosphate Buffered Saline

PEG : Polyéthylène Glycol

RABV : Classic rabies virus

rpm: Rotations per minute

IPTG: Isopropyl β-D-1-thiogalactopyranoside

SDS : Sodium Dodécyl Sulfate

TEMED : Tétraméthyl Ethylène Diamine

TMB : 3 ,3', 5,5'Tétraméthyl benzidine

TNF : Facteur de nécrose des tumeurs

ua : Absorbance unit

UI : International unit

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How to cite this article:
Gasmi, M. ; Rahma,Z ; Williams-Hooker, R.; Sharma, Aastha . Production and Characterization of Recombinant Multi-Epitope G Glycoprotein of Rabies Virus Med. Pharm. J. 2023;2(3):134- 148.
DOI: 10.55940/medphar202351
Available from: http://pharmacoj.com/ojs/index.php/Medph/article/view/51