UDC 575.
https://doi.org/10.2298/GENSR1803919R
Original scientific paper

THE STUDY ON THE STIMULATION OF THE IMMUNE SYSTEM IN THE INFUSED BALB/c MICE BY PCDNA3.1(-)-*FLAA* RECOMBINANT VECTOR AGAINST *Helicobacter pylori* INFECTION USING MOLECULAR TECHNIQUES

Behrouz RAMAZANI-DEHNAVI[1], Abbas DOOSTI[2*], Mohammad-Saeid JAMI[1,3]

[1] Department of Biology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[2] Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[3] Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

Ramazani-Dehnavi B., A. Doosti, M.S. Jami (2018): *The study on the stimulation of the immune system in the infused BALB/C mice by pCDNA3.1(-)-flaA recombinant vector against Helicobacter pylori infection using molecular techniques*.- Genetika, Vol 50, No.3, 919 - 932.

Helicobacter pylori, a Gram-negative flagellated microaerophilic bacterium, is associated with inflammation of the stomach, duodenal and gastric ulcer disease, and gastric cancer. This bacterium infects almost half of the world's population stomachs. One of this pathogen's immunogenic genes is the *flaA* gene that can stimulate the host immune system. In our previous study, the immune response in pCDNA3 1(-)-*flaA* infused BALB/c mice against *H. pylori* infection was investigated using quantitative real-time PCR (q-RT-PCR). After preparation of pCDNA3 1(-)-*flaA* recombinant plasmid at large-scale, the mice were infused in the hip muscle by a recombinant vector with or without chitosan nanoparticles. The pcDNA3 1(-) was used as the negative control. The blood and

Corresponding author: Abbas Doosti, Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran, Postal box: 166; Tell: +98-38-33361001. Fax: +98-38-33361001; E-mail: biologyshki@yahoo.com

tissue specimens of each mouse were collected at different times. The expression levels of cytokine genes (including *IL-2*, *IFNγ*, *IL4*) and the internal control gene were evaluated in peripheral blood cells using a q-RT-PCR method. Also, the *flaA* gene expression in mice muscle was measured at 15, 30, and 45 days after the last injection. In infused mice by pcDNA3 1(-)-*flaA*, the *IL-2* and *IFNγ* genes were increased statistically (p <0.001) and *IL4* was significantly decreased (p <0.001). Moreover, the expression of *flaA* gene in mice muscles was decreased by passing the time. Furthermore, the infused mice by pcDNA3 1(-)-*flaA* + nanoparticles showed a better immune response because of alteration in *IL4* expression. Our findings in infused mice by pcDNA3 1(-)-*flaA* suggested that the expression level of *IL-2* and *IFNγ* were increased compared to the IL-4 via simulation of Th1. Also, the expression of the *flaA* gene in tissue samples was decreased 45 days after the last injection. Therefore, it can be concluded that the pcDNA3.1(-)-*flaA* recombinant vector together with chitosan nanoparticles has the ability to stimulate the immune system and it can be investigated as a cost-effective method to control the *H. pylori* disease in a human in further studies.

Keywords: *Helicobacter pylori*, *flaA* gene, immune stimulation, cytokines gene expression

INTRODUCTION

One of the most important factors in gastrointestinal infection and gastric malignancy is *Helicobacter pylori* (*H. pylori*) that can cause gastric adenocarcinoma, peptic ulcers, gastrointestinal tract inflammation, and stomach cancer (KIM *et al.*, 2011; MCDONALD *et al.*, 2015). *H. pylori* (previously *Campylobacter pylori*) is a Gram-negative, rod-shaped, microaerophilic bacterium with flagella that can survive in the stomach by producing important enzymes like oxidase, catalase, and urease (PARK *et al.*, 2011; FRIRDICH *et al.*, 2017). The prevalence of *H. pylori* in early childhood is 30%-50% and the infection rate varies between different regions and increases (more than 90%) in the asymptomatic adult population. In developing countries, the infection rate in the adult population is high (approximately 80%) (TESTERMAN and MORRIS, 2014; TRINDADE *et al.*, 2017).

Several factors including flagellate, adhesions, urease, cag cytotoxin-associated gene A (CagA), and vacuolating cytotoxin (Vac) are involved in pathogenicity of *H. pylori* (BUTI *et al.*, 2011; MESSING *et al.*, 2014). *H. pylori* flagella play a key role in this pathogen's establishment, colonization, and infection in the stomach mucosa (GU, 2017). More than about 50 proteins involve in the expression, secretion, and assembly of the flagellum in this bacterium that at least 20 of them are parts of structural components of the basal body, the hook and the filamentous (KAO *et al.*, 2016). The flagellum is composed of two types of flagellin including FlaA (1530 nucleotides, 53 KDa) and FlaB (54 KDa) (YAN *et al.*, 2003; SADEGHI and DOOSTI, 2017). Amino acid homology between *flaA* and *flaB* subtypes is 58% identical and both genes are essential for the full motility and sustainability of the contamination in gastric mucosa (FABER *et al.*, 2016). FlaB is a larger and scattered strand protein exclusively near the source. *H. pylori* flagellin is an important virulent factor in the pathogenesis of gastric mucosal and is considered as one of the most important stimulators of the immune system and acts as a ligand for toll-like receptor 5 (TLR5) of the host cells (YAN *et al.*, 2003; SADEGHI and DOOSTI, 2017). FlaA protein is the predominant subtype and reduces TLR5 internal activity in this pathogen so it might help the

bacterium to escape from the host immune response and causes continuous bacterial colonization (SADEGHI and DOOSTI, 2017; SEO *et al.*, 2017).

According to the US Food and Drug Administration (FDA) the regular treatment against *H. pylori* infection is a 7-day triple antibiotic regimen containing lansoprazole, amoxicillin, and clarithromycin (LAC) as the first line of treatment (YAKOOB *et al.*, 2014; SAPMAZ *et al.*, 2014).

The increasing prevalence of *H. pylori* infection, antibiotics side effects and resistance, failure in eradication of infection, *H. pylori* persistence in human host, having no effect on inactive bacteria, drug interactions and high costs for antibodies production, have made it essential to achieve an alternative therapy or preventive approach such as vaccination (WHEELDON *et al.*, 2005; NGOYI *et al.*, 2015). Recombinant vaccines such as DNA vaccines, compared to other therapeutic methods and vaccines like live-attenuated or killed vaccines are inexpensive and the production of recombinant proteins is affordable. Moreover, the product is pure, stable at room temperature, high specific, stimulating both the cellular and humoral immune responses, easily prepared and can reduce the risk of infection (TOBER *et al.*, 2014; DOOSTI *et al.*, 2015). Considering the present treatment problems, the stimulation of the immune system by *flaA* gene, as a recombinant DNA vaccine, was evaluated against *H. pylori* infection in the infused BALB/c mice DNA using q-real-time RT-PCR in this study.

MATERIALS AND METHODS

pCDNA3.1(-)-flaA Recombinant vector preparation

H. pylori flaA gene (1545 bp) was synthesized and cloned into 5427 bp empty pCDNA3.1(-) plasmid (Invitrogen, San Diego, CA), as a control plasmid, by Generay Biotech Co., Ltd. (Shanghai, China) company and obtained from the SADEGHI and DOOSTI, 2017 study. pCDNA3.1(-)-*flaA* recombinant vector was prepared for injection into BALB/c mice. The lyophilized stock of Top10F' *E. coli* was purchased from Pasteur Institute (Tehran, Iran) and was used as a transformation host. This bacterial strain was cultured on Luria-Bertani (LB) agar plate to obtain a single colony. The colony was cultured into 5 mL of LB broth containing 1% tryptone, 0.5% yeast extract and 0.5% sodium chloride in sterile conditions and then it was placed in a shaking incubator overnight at 37°C.

Vector transformation

The recombinant pCDNA3.1(-)-*flaA* was transformed into competent *E. coli* Top10F' strain using $CaCl_2$ (0.1 M) and heat shock (90 seconds at 42°C) treatments. Transformation confirmation was done by colony-PCR and the selected colony was cultured in 5 mL LB broth containing 100 µg/mL of ampicillin and incubated overnight at 37°C and 200 rpm shaking. Plasmids were isolated from a sample of culture using miniprep plasmid purification kit (Qiagen; Valencia, CA, USA). To confirm the transformation, the enzymatic digestion with *EcoRV* and *KpnI* restriction enzymes, polymerase chain reaction (PCR), and sequencing were performed on purified plasmids. A Maxiprep plasmid purification kit (Qiagen, Valencia, CA) was used for large scale extraction for vector injection in laboratory animals according to the manufacturer's instructions. The electrophoresis on a 2% agarose gel in 1X TBE buffer at constant voltage of 80 V using 1 kb molecular marker was done for 30 min and also the optical density (OD) measurement at a wavelength of 260/280 nm using Thermo Scientific™ NanoDrop 2000 (Wilmington, DE, USA) was done to verify the extracted plasmids' quality and quantity

(recombinant vector and empty pCDNA3.1) according to the method described by SAMBROOK and RUSSELL, 2001. After electrophoresis, the gel was stained by 0.1% ethidium bromide (0.4 g/mL) and the images were taken in UVIdoc gel documentation systems (Uvitec, UK).

Conventional PCR assay

The conventional PCR was done for confirmation of *flaA* gene presence in purified plasmids. The specific oligonucleotide primers were designed using the Primer Express Software (version 2.0; Applied Biosystems) and the Basic Local Alignment Search Tool (BLAST) from the GenBank database (Table 1).

The amplification mixture was carried out in a final volume of 25 µL per 0.2 mL micro-tubes. The PCR reaction mixture contained 10 pmol of each forward and reverse primers, 2.5 µL 10× PCR buffer, 1 µL dNTP mix (10 mM), 5 µL $MgCl_2$ (25 mM), 50 ng of plasmid DNA, 1.25 U of *Taq* DNA polymerase (all reagents were purchased from Invitrogen Life Technologies, Carlsbad, CA,USA). The amplification program included an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, extension at 72°C for 1 min, with a final extension at 72°C for 10 min and hold at 4°C in a Master Cycler Gradient device (Eppendorf AG, Hamburg, Germany). PCR products were separated using 2% agarose gel electrophoresis according to the method mentioned above.

Preparation and in vitro characterization of chitosan nanoparticles

Chitosan nanoparticles were prepared according to the ionotropic gelation of chitosan with pentasodium tripolyphosphate (TPP) anions described by ELWERFALLI *et al.*, 2015 and VAEZIFAR *et al.*, 2013. Acetic acid solution (1% or 2 mg/mL) was prepared and chitosan powder (Sigma-Aldrich, USA) was dissolved in it under magnetic stirring at 1000 rpm and incubated at room temperature for 24 hours. The pH was then adjusted to 5.5 using 0.5 M NaOH. This solution was filtered through a 0.45 µm filter and then 0.7 mg/mL of sodium tri-polyphosphate (TPP) solution was prepared and after filtration 20 mL of it was added dropwise and very slowly to 50 mL of an aqueous acetic acid solution containing chitosan and was kept under constant agitation on magnetic stirrer at 1000 rpm for 1 hour. Finally, the nanoparticles suspension was centrifuged at 14000 rpm (4°C) for 15 min and the supernatant was powdered using freeze-dryer (Virtis Advantage Plus freeze-dryer; SP Scientific, Warminster, USA).

The size of nano-scale particles, size distribution, and zeta potentials of chitosan nanoparticles was measured using a Malvern Nano ZS90 Zetasizer Nanoseries system (Malvern Instruments, Malvern, UK). Equal volumes of each solution including 1% chitosan nanoparticles and plasmid (2000 µg/mL in PBS) was mixed well by pipetting up and down and incubated for 1 hour at 55°C.

Ethics Statement and DNA vaccines in animals

This study was approved by the Ethical Committee and Research Deputy of the Islamic Azad University of Shahrekord Branch, Iran on September, 25th 2016. For immunization a total of 63 5 weeks old female BALB/c mice were divided into three groups (each group contains 21 mice) including recombinant plasmid-immunized group (pCDNA3.1(-)-*flaA*), recombinant plasmid+ chitosan nanoparticles group, and pcDNA3.1(-) as a control group. 1000 µg/mL pcDNA3.1(+) vector (empty plasmid), 1000 µg/mL of pCDNA3.1(-)-*flaA* (dissolved into PBS), and 1000 µg of pCDNA3.1(-)-*flaA* + nanoparticles were prepared separately and 100 µL of each

DNA vaccine was used for intramuscular (IM) injection into the quadriceps muscle of each mouse on days 0, 7, and 15. At each injection stage, 7 mice of each group were killed and 1.5 mL of blood samples were taken directly from the heart by syringe aspiration and was collected into ethylenediaminetetraacetic acid (EDTA) tubes. Then, the skin of the mouse thigh (exactly at the site of the injection) was removed and used for RNA extraction in further tests.

Quantitative real-time PCR (q-RT-PCR) assay

100 mg of each tissue sample or 100 µL of blood specimens' buffy coat were used for RNA purification using the TRIzol reagent according to the manufacturer's protocol (Invitrogen Life Technologies). RNA concentration was measured by the ratio of absorbance at 260 and 280 nm and RNA from each sample was reverse-transcribed to cDNA using SuperScript ® III (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. The sequences of designed primers by Gene Runner software version 3.05 (Hastings Software Inc. Hastings, NY, USA) used for q-RT-PCR are shown in Table 1.

Table 1. Primer sequences used for q-RT-PCR

Primers	Sequence	Annealing temperature (ºC)	Product length (bp)	Accession number
	β-actin	67 F: 5′-GCTGTCCCTGTATGCCTCTGGTC-3′ R: 5′-ATGTCACGCACGATTTCCCTCTC-3′	219	NM_007393
IL-2	F: 5′-TGCCTAGAAGATGAACTTGGACCTC-3′ R: 5′-GGCTTGTTGAGATGATGCTTTGAC-3′	65	229	BC116845
4-IL	′3-TCACAGGAGAAGGGACGCCATG-F: 5′ ′3-TGGACTTGGACTCATTCATGGTGC-R: 5′	67	246	IFNγ64F: 5′-GCCTAGCTCTGAGACAATGAACG-3′NM_021283 R: 5′-GCCAGTTCCTCCAGATATCCAAG-3′
flaA	F: 5′-TAAAATCGGTCAGGTTCGTATCG-3′ R: 5′-TGTGGTGATAACGCTCGCATAAG-3′	65	217	EU400216188 M28621

The cytokines gene expression levels (including *IL-2*, *IL-4*, and *IFNγ*) compared to *β-actin* (reference gene) and *flaA* as a target gene of recombinant vector were measured by the Corbet Rotor-Gene 6000 rotary analyzer (Corbett, Australia) and the cycle of threshold (Ct) values were determined by the $2^{-\Delta\Delta Ct}$ relative quantitative method. Each q-RT-PCR reaction was done in a final volume of 25 µL containing 5 µL cDNA template (50 ng), 12.5 µL 1× SYBR

Green real-time PCR master mix (Toyobo, Osaka, Japan), 1 μL of each primer (2 μM), and 5.5 μL distilled water. The thermal cycling program was initial denaturation at 95˚C for 1 min, followed by 45 cycles of denaturation at 95˚C for 10 sec, annealing at variable temperatures for 30 sec, and extension at 72˚C for 30 s. At the end of each reaction, a melting-curve analysis of machine was set from 55˚C to 95˚C at a transition rate of 0.1˚C/s to monitor primer dimers or non-specific product formation.

Statistical analysis

Each experiment was repeated at least three times. The Rest 2009 software was used to calculate gene expression ratios. All analyses were conducted using Social Sciences software (SPSS, Inc., Chicago, IL, USA) version 20 followed by independent T-test to investigate the significance of the data. All data were considered as mean + SEM at a significant level of $p < 0.05$.

RESULTS

Confirmation of produced recombinant plasmid

The recombinant pCDNA3.1(-)-*flaA* was constructed and transformed into competent *E. coli* cells successfully. The accuracy of the pCDNA3.1(-)-*flaA* transformation was verified by PCR reaction and enzymatic double digestion. PCR method proved the presence of the target *flaA* gene in final construct and double restriction enzyme digestion pattern by *EcoRV* and *KpnI* in pCDNA3.1(-)-*flaA* recombinant vector on agarose gel electrophoresis showed two fragments including 1545 bp fragment for *flaA* gene and 5427 bp related to the linear pcDNA3.1(-) vector (Figure 1).

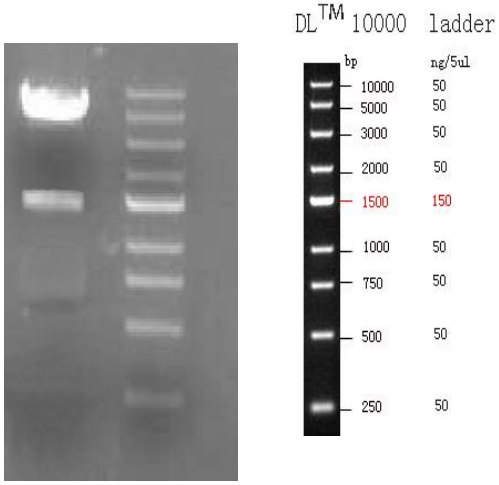

Figure 1. Confirmation of the presence of *flaA* gene in pCDNA3.1(-)-*flaA* recombinant vector by enzymatic digestion on 2% agarose gel electrophoresis

Physicochemical properties of chitosan nanoparticles

The physicochemical properties of chitosan nanoparticles including particle size, particle charge, and dispersion index were measured using dynamic light scattering (DLS; Malvern Zetasizer Nano ZS, ZEN 3600) at 25°C at a wavelength of 633 nm and it was found that 98.8% of these particles had a size of 132.6 nm, size distribution of 36 and zeta potential of 59.3 mV (Figure 2). Furthermore, a regular, spherical and homogeneous with smooth edges appearance of chitosan nanoparticles was observed by scanning electron microscope (SEM).

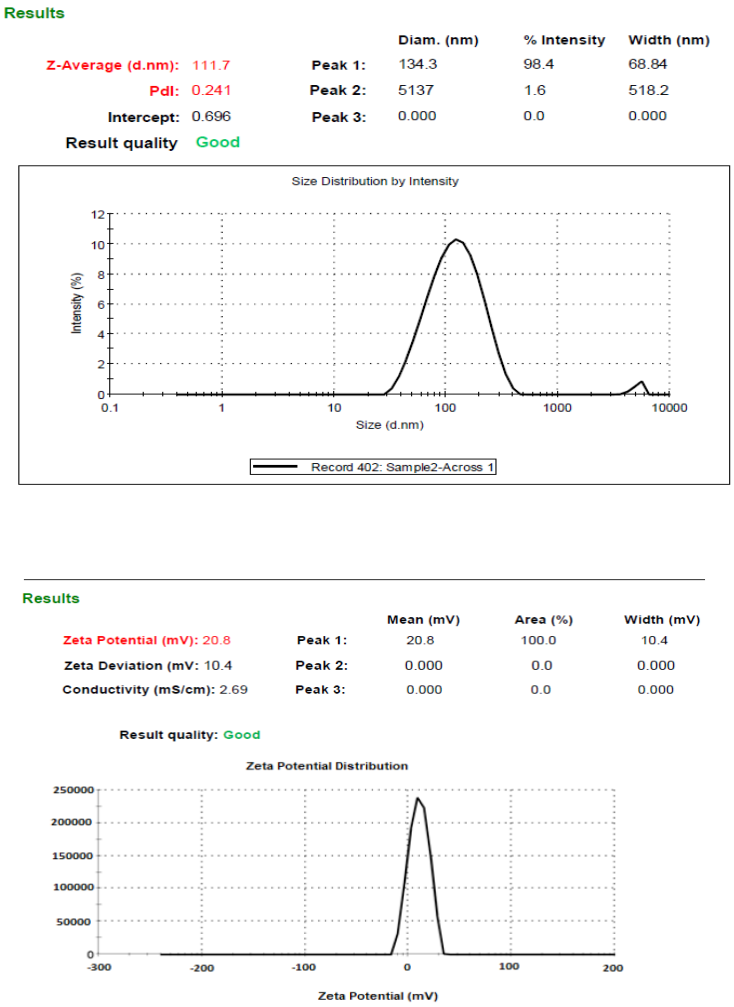

Figure 2. Zeta-potential distribution (left) and particle size (right) of chitosan nanoparticles

Q-real-time PCR analysis

The quantitative gene expression analysis using real-time RT-PCR and REST 2009 software showed that the expression levels of *IL-2* and *IFNγ* genes in the infused group by pcDNA3.1(-)-*flaA* were increased statistically (p <0.001) while the *IL4* expression was significantly decreased (p <0.001) (Figure 3).

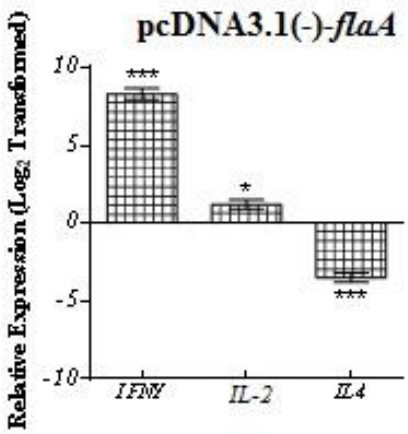

Figure 3. The changes of the cytokines expression after injection of pcDNA3.1(-)-*flaA* in the infused mice

The comparison between cytokine genes expression level in infused mice by pcDNA3.1(-)-*flaA* and pcDNA3.1(-)-*flaA* containing chitosan nanoparticles showed that *IL-2* and *IFNγ* genes were not expressed significantly different in these two groups (p >0.05), while the difference in *IL4* expression was meaningful (p <0.05) (Figure 4).

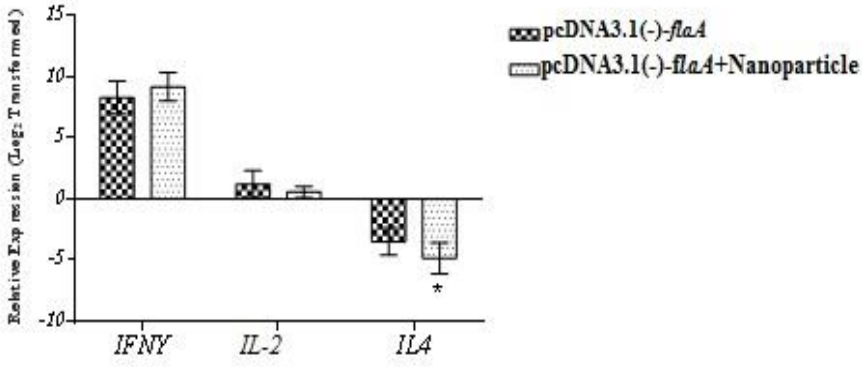

Figure 4. The alteration of cytokine genes expression after injection of pcDNA3.1(-)-*flaA* group compared to pcDNA3.1(-)-*flaA* + nanoparticle

The *flaA* gene expression level in tissue samples of infused mice by pcDNA3.1(-)-*flaA* and pcDNA3.1(-)-*flaA* + nanoparticle in different times after the last injection, including day 15 compared to days 30 and 45, and day 30 compared to day 45 was significantly different (p <0.001) (Figure 5).

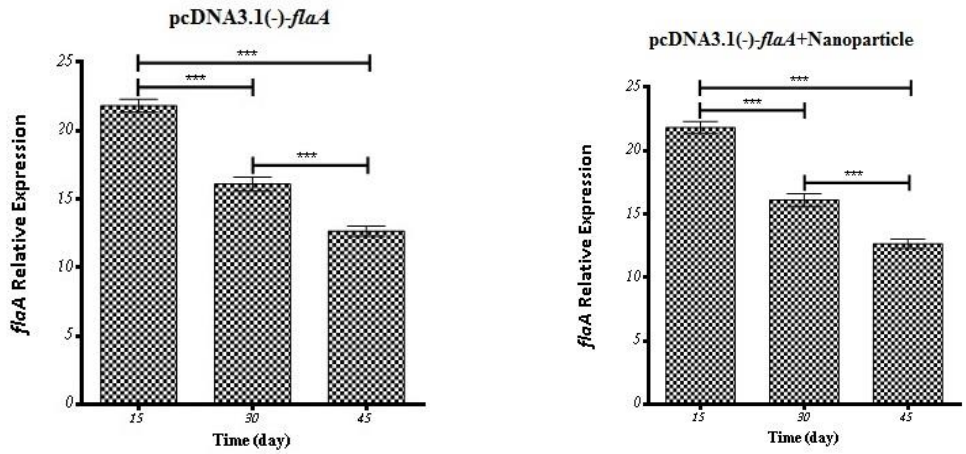

Figure 5. Analysis of *flaA* gene relative expression in infused BALB/c mice by pcDNA3.1(-)-*flaA* (left) and pcDNA3.1(-)-*flaA* + nanoparticle (right) tissue at day 15 compared to days 30 and 45 after the last injection

Furthermore, the *flaA* gene expression in infused mice by pcDNA3.1(-)-*flaA* + nanoparticle compared to pcDNA3.1(-)-*flaA* at days 15 and 45 after the last injection was not significantly different (p >0.05), while the expression changed at day 30 (p <0.001) (Figure 6).

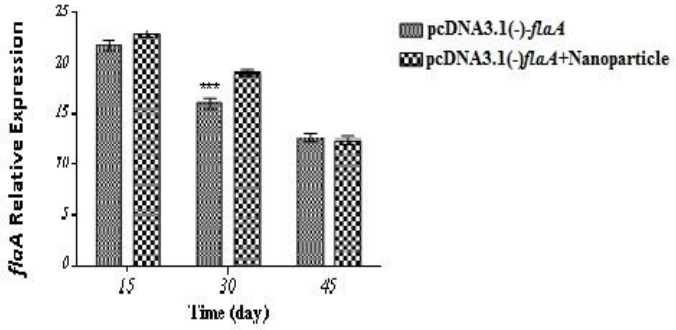

Figure 6. The teration of *flaA* gene expression level in the tissue samples of infused mice by pcDNA3.1(-)-*flaA* compared to the nanoparticle group at 15, 30, and 45 days after the last injection

DISCUSSION

H. pylori is one of the most common infectious agent in human societies and it has a difficult clinical diagnosis. Furthermore, this bacterium can cause chronic and active gastritis, duodenal ulcer and stomach ulcer (MÓNICA and ARMELLE, 2013). Despite many efforts, an effective vaccine against this pathogen has not been developed yet. The most significant characteristic of a vaccine, especially DNA vaccine, is its antigenicity and ability to stimulate humoral and cellular immune system (ANDERL and GERHARD, 2014; WANG *et al.*, 2014). One of the most important antigens of *H. pylori* is a flagellar antigen. This pathogen has 5 to 7 flagella, which give the bacterium the abilities like mobility and colonization. Also, mutations in flagellum coding gene reduce the pathogenicity of the bacteria (GU, 2017; ZAREI *et al.*, 2017). Studies show that *FlaA*, as well as *FlaB*, may be used as a candidate for *H. pylori* antigen vaccine and because there is not an appropriate vaccine to control this pathogen (CHEN *et al.*, 2016), the present study was performed to evaluate the variation of gene expression and the efficiency of recombinant pCDNA3.1(-)-*flaA* DNA vaccine to stimulate the immune system in the infused BALB/c mice against *H. pylori* infection using molecular techniques. The pCDNA3.1(-)-*flaA* and chitosan nanoparticles were prepared at an appropriate concentration by ionic gelation method and were injected into BALB/c mice's hip muscle. Each infused animal's blood and tissue samples were collected at 15, 30, and 45 days after the last injection. After total RNA extraction and cDNA synthesis, the expression levels of cytokine genes including *IFNγ*, *IL-2*, and *IL-4* were evaluated by q-real-time RT-PCR for investigation of *flaA* (as a target gene of recombinant plasmid) effects on immune system stimulation against *H. pylori* infection. The results showed that the expression level of *IFN-γ* and *IL2* was increased (p <0.001), while, *IL4* expression was significantly decreased (p <0.001). Also, the expression levels of cytokine genes in pcDNA3.1(-)-*flaA* compared to pcDNA3.1(-)-*flaA*+chitosan nanoparticles showed that *IL-2* and *IFNγ* genes were not expressed significantly different (p >0.05) but *IL4* expression in the pcDNA3.1(-)-*flaA* + nanoparticle group was meaningfully different (p <0.05). Also, the expression level of *flaA* gene was decreased significantly in the injection site of tissue on days 12, 30, and 45 after injection and *flaA* gene expression was significantly different (p <0.001) in infused mice by pcDNA3.1(-)-*flaA* + nanoparticle compared to pcDNA3.1(-)-*flaA* on day 30.

Difficulties in the diagnosis of gastric disease caused by *H. pylori* infection at early stages, the antibiotic resistance, and the prevalence of disease at high level have led to perform many studies on development of specific and new therapies such as designing a proper vaccine like DNA vaccine against this infection (KUSTERS *et al.*, 2006; WROBLEWSKI *et al.*, 2010).

Mori and colleagues (MORI *et al.*, 2012) investigated the cloning and gene expression of *H. pylori flaA* gene combined with *E. coli flaC* gene as an adjuvant and adjuvant and antigen expressed together. The application of *flaA* gene in their study was similar to our work, which indicates the high importance of this gene as a candidate for vaccine therapy against *H. pylori*. A team of researchers cloned and expressed the *Vibrio parahaemolyticus flaA* gene and suggested that the recombinant FlaA protein could be used as a recombinant vaccine or in structural and functional studies and confirmed our results (YUAN *et al.*, 2010). Zhang and colleagues studied the production of *UreB* oral (Urease B) vaccine against *H. Pylori* in 2014. They found that mucosal recombinant vaccination leads to the production of enormous anti-UreB antibodies and increased levels of Ifn-G, Il-4, and Il-17 cytokines (ZHANG *et al.*, 2014). In a review study by MIRZAEI *et al.*, 2017, the most known vaccine candidates and multi-component vaccines including single (AhpC, BG, CagA, KatA, Fla, Hsp, HWC, Lpp, LPS, NAP, OMP, OMV, SOD,

Tpx, Urease, VacA) against *H. pylori* infection was discussed. In another study by Yang *et al.* (2015) the oral intake effects of multi-epitope CTB-UreI-UreB (BIB) vaccine against *H. Pylori* was examined. They observed a strong immunity in BIB vaccinated BALB/c mice compared to adjuvant or PBS vaccinated mice against this pathogen. SONG *et al.* (2015) made a new vaccine called CTB-UE-CF (CCF), and after it's injection into mice the presence of *H. pylori* was evaluated using Real-Time PCR. Their research indicated a relative immunity against *H. pylori* infection in tested animals. In 2017, Sjökvist and colleagues examined the effect of adjuvant auxiliary along with the vaccine and prescribed the vaccine in two ways including either sublingually or in the stomach. Their results showed that adding an adjuvant to the vaccine after sublingual vaccination activates dendritic cells, especially in the neck lymph nodes, and it leads to increased level of IL-17A, IFN-C and TNF-A cytokines (SJÖKVIST *et al.*, 2017). In a new study by SADEGHI and DOOSTI, 2017 the *flaA* gene transformed into pTZ plasmid and subcloned into the pcDNA3.1(-) expression vector and then this construct transformed into CHO cells by electroporation, and the gene expression was investigated by SDS-PAGE. They showed that this generated gene construct can express the *flaA* product in eukaryotic systems. Also, our q-real-time RT-PCR findings showed that pcDNA3.1(-)-*flaA* construct alone or combined with nanoparticles can induce the immune system.

Most of these researches have focused on *flaA* gene cloning and expression of *H. pylori* or another bacterial sample. These studies suggested that *flaA* gene is one of the most important virulent factors in this bacterium and its protein or recombinant protein derived from it can be used in vaccine researches.

CONCLUSIONS

According to our study results, an effective immune response to the pCDNA3.1(-)-*flaA* construct was observed in the infused mice blood and muscle. Also, the expression level of *flaA* gene was decreased over time and studied cytokines expression level changed significantly (*IL-2* and *IFNγ* genes were increased while the *IL4* expression was decreased). Moreover, recombinant vector as a DNA vaccine combined with chitosan nanoparticles can increase the effectiveness of the vaccine by altering *IL4* expression in pcDNA3.1(-)-*flaA* + nanoparticle infused BALB/c mice. Due to our findings about immune system stimulation by pcDNA3.1(-)-*flaA* construct, this construct could be useful in future studies about the eukaryotic system researches, human studies, and biomarker discovery and preclinical drug testing in animal models.

ACKNOWLEDGEMENTS

This article was obtained from the MSc thesis and the authors would like to extend their sincerest thanks and appreciation to the Research Deputy of the Islamic Azad University of Shahrekord Branch and to all staffs in the Biotechnology Research Center for their cooperation.

Received, January 02nd, 2018

Accepted August 18th, 2018

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STUDIJA O STIMULACIJI SISTEMA IMUNOG SISTEMA KOD BALB / c MIŠA POMOĆU PCDNA3.1 (-) - FLAA RECOMBINANTNOG VEKTORA PROTIV *Helicobacter pilori* INFEKCIJE UPOTREBOM MOLEKULARNIH TEHNIKA

Behrouz RAMAZANI-DEHNAVI[1], Abbas DOOSTI[2*], Mohammad-Saeid JAMI[1,3]

[1] Departman za biologiju, Fakultet za osnovne nauke, Shahrekord ogranak, Islamski Azad Univerzitet, Shahrekord, Iran
[2] Biotehnološki istraživački centar, Shahrekord ogranak, Islamski Azad Univerzitet, Shahrekord, Iran
[3] Ćelijski i molekularni istraživački centar, Institut za osnovne zdravstvene nauke, Shahrekord Univerzitet medicinskih nauka, Shahrekord, Iran

Izvod

Helicobacter pylori, Gram-negativna mikroaerofilna bakterija, povezana je sa zapaljenjem želuca, bolestima duodenalnog i želudačnog ulkusa i karcinomom želuca. Ova bakterija zaražava stomak skoro polovine svetske populacije. Jedan od imunogenih gena ovog patogena je gen flaA koji može stimulisati imunološki sistem domaćina. U našoj prethodnoj studiji, imunološki odgovor u pCDNA3.1 (-) - flaA inficiranom BALB/c mišu na *H. pylori* infekciju, ispitivan je pomoću kvantitativnog RT-PCR-a (q-RT-PCR). Nakon pripreme pCDNA3.1 (-) - flaA rekombinantnog plazmida, miševi su infuzionisani u mišiće nogu rekombinantnim vektorom sa ili bez nanočestica. pcDNA3.1(-) je korišćena kao negativna kontrola. Uzorci krvi i tkiva svakog miša prikupljeni su u različito vreme. Nivo ekspresije citokin gena (uključujući *IL-2*, *IFNγ*, *IL4*) i interne kontrole bili su ocenjeni u perifernim krvnim ćelijama metodom q-RT-PCR. Takođe, ekspresija flaA gena u mišićima miševa merena je na 15, 30 i 45 dana nakon poslednje injekcije. Kod miševa gde je ubačen pcDNA3.1(-)-*flaA*, *IL-2* i *IFNγ* geni su se značajno statistički povećali (p <0.001), a *IL4* se značajno smanjio (p <0.001). Takođe, ekspresija *flaA* gena u mišićima miša je opadala s vremenom, dok su miševi u koje su ubačene pcDNA3.1(-)-*flaA* + nanočestice, pokazali bolji imuni odgovor zbog izmene u ekspresiji *IL4*. Na osnovu rezultata, može se zaključiti da rekombinantni vektor pcDNA3.1(-)-*flaA* zajedno sa nanočesticama stimuliše imuni sistem i može se ispitati u budućim proučavanjima kao isplativ metod za kontrolu *H. pylori* kod ljudi.

Primljeno 02.I.2018.
Odobreno 18. VIII. 2018.