Use of N-trimethyl chitosan for intranasal delivery of DNA encoding M2e-HSP70c in mice

Dabaghian, M.[1], Ebrahimi, S.M.[2], Nikbakht, Gh.R.[1*], Tebianian, M.[3], Rezaei Mokaram, A.[3], Iman, M.[4], Tavangar Ranjbar, A.R.[5]

[1]*Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2]*Research Center of Virus and Vaccine, Baqiyatallah University of Medical Sciences, Tehran, Iran*

[3]*Department of Biotechnology, Razi Vaccine and Serum Research Institute (RVSRI), Karaj, Iran*

[4]*Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

[5]*Department of Virology, Tehran University of Medical Sciences, Tehran, Iran*

Key words:
Influenza, M2e, HSP70, N-trimethyl chitosan (TMC), Intra nasal delivery

Correspondence
Nikbakht, Gh.R.
Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Tel: +98(21) 61117057
Fax: +98(21) 66427517
Email: nikbakht@ut.ac.ir

Received: 27 February 2013
Accepted: 21 May 2013

Abstract:

BACKGROUND: Influenza outbreak has become a great life-threatening disease in the world. Nasal vaccines can induce systemic IgG and mucosal IgA antibody responses, which establish two layers of immune defense against the infectious pathogens like influenza. Mucosal vaccines must overcome several limitations, including the mucociliary clearance and inefficient uptake of soluble antigens. Therefore, nasal vaccines require potent adjuvants and delivery systems. **OBJECTIVES:** In this study we evaluated the effect of N-trimethyl chitosan (TMC) as a potent vehicle for DNA encoding M2e/HSP70c in order for intranasal administration in mice. **METHODS:** Ectodomain of the conserved influenza matrix protein 2 (M2e), which has been found to induce heterosubtypic immunity, was fused to HSP70359-610 or C-terminus of *Mycobacterium tuberculosis* HSP70 (HSP70c) in pcDNA 3.1 vector (pcDNA/M2e-HSP70c) and then encapsulated into a derivative of chitosan, N-trimethyl chitosan (TMC). After encapsulation of the plasmid, physical properties of the particles were investigated using Zetasizer® 3000 the particles were then administered through the intranasal delivery in BALB/c mice. **RESULTS:** It was found that the particles had a size ranging between 90-120nm and positive surface charge. The intranasal immunization with M2e-HSP70c+TMC in BALB/c mice significantly induced higher M2e specific IgG than those induced in control groups (pcDNA/M2e-HSP70c without TMC, pcDNA/M2e, bearing M2e alone, and PBS). **CONCLUSIONS:** The present study showed that the encapsulation of M2e/ HSP70c into N-trimethyl chitosan (TMC) could strongly induce the humoral immune response against the M2e-HSP70c plasmid without lowering the adjuvant efficacy of HSP70c.

Introduction

Influenza has remained a serious widespread threat to the human and animal population. Vaccination is the best prophylactic strategy for controlling the influenza infection. The current licensed vaccines (i.e. synthetic virus-like particles, inactivated or live-attenuated whole virus, split and subunit vaccines) are based on the immune response against

membrane glycoproteins, HA and NA. The great capacity of these glycoproteins for the mutation and reassortment of virus genes within host animals infected by multiple strains of influenza, often lead to the emergence of new virulent strains (Hay et al., 2004).

The extracellular domain of the M2 protein (M2e) is considered as an attractive target for developing a universal influenza vaccine. M2e consists of 24 amino acids which remain highly conserved across all influenza isolates (Neirynck et al., 2007; De Filette et al., 2005). Notably, M2e is not very immunogenic and the use of M2e alone for developing a candidate vaccine cannot induce a strong immune response. Thus, different adjuvants have been utilized to increase the immunogenicity of the M2e protein.

HSP70 belongs to the heat shock protein family. The adjuvant effects of HSP70 have been shown for different microorganisms like *mycobacterium tuberculosis* (Lowrie et al., 1999) and plasmodium (Qazi et al., 2005). The HSP70 molecule consists of N-terminal and C-terminal parts. The C-terminal domain (HSP70359-610) is the peptide-binding part of the HSP70 with adjuvant properties (Rafati et al., 2008). This part of HSP70 can induce the activation of APCs and NK cells through receptors like CD91 and LOX1. Moreover, the interaction of HSP70 with TLR2 and TLR4 results in the production of proinflammatory cytokines. These properties lead to the use of HSP70 as a potent adjuvant for DNA- and protein-based vaccines (Ebrahimi et al., 2010; Suzue et al., 1996).

The plasmid encoding extracellular domain of the M2 protein (M2e) and the C-terminal domain of *Mycobacterium tuberculosis* HSP70 (HSP70 359-610 or HSP70 C-terminal) could induce a strong humoral immune response when injected subcutaneously in mice (Zabeh et al., 2012).

Mucosal immunization via the nasal route is an attractive alternative to parenteral immunization (Suzue et al., 2005). The mucosal route is a way that infectious agents like Influenza virus use for entering the body, so it is the first line of defense against such particles. Particles taken up by nasal epithelia and nasal-associated lymphoid tissue (NALT) can lead to the induction of both mucosal and humoral immunity (Almeidaet al., 1993; Alpar et al., 1994; Pawar et al., 2010). Several methods and polymers have been used to overcome the limitations of mucosal routs for the antigen delivery such as using viral (like HBc) or non-viral (like PLGA) vehicles (Turley et al., 2011; Pawar et al., 2010). In recent years different studies have been conducted in which the chitosan and its derivatives were used as a delivery system for DNA and protein antigens (Roy et al., 1999).

Chitosan is a biodegradable, biocompatible, non-toxic, and muco-adhesive polysaccharide. It can be produced by the deacetylation of the naturally acquired chitin (Illum et al., 1998). Because of the mucociliary clearance mechanism that removes soluble antigens from the nasal cavity, any particle that is administered through the nasal route can only remain for only a short time (Soane et al., 1999; Soane et al., 2001). To overcome this limitation, a water soluble derivative of chitosan, N-trimethyl chitosan (TMC), was used as a carrier for the M2e-HSP70c plasmid via the nasal route. In this study we investigated the effectiveness of TMC in promoting the efficacy of M2e-HSP70c DNA based vaccine through intranasal administration.

Materials and Methods

Preparation of recombinant plasmids: The confirmed recombinant pcDNA 3.1 (+) plasmid bearing separate genes sequence of M2e-HSP70c or M2e (signated as pcDNA/M2e-HSP70c and pcDNA/M2e) were constructed, purified, and then confirmed according to our previous study (Zabeh et. al., 2012). In order to evaluate immune responses against them, the recombinant vectors were then purified by endotoxin free plasmid Mega Kit (Qiagen, USA) according to the manufacture's instruction.

Preparation of chitosan-DNA particles: The N-trimethyl chitosan (TMC) used in this study was provided by Baqiyatallah Medical Sciences University, Iran. The TMC solution (2mg/mL in water, pH 6) and a DNA solution (100µg/mL in 5mM of NaAc solution) were preheated to 50-55°C separately. An equal volume of both solutions were quickly mixed while vortexing. The chitosan-DNA particles were incubated at room temperature for 30 min before characterization and transfection. The particle size and zeta-potential of the chitosan-DNA were measured on a Zetasizer® 3000 (Malvern Instruments, UK).

Immunization: Four groups of six to eight-week-old female BALB/c mice (twenty mice per group) were immunized with pcDNA/M2e-HSP70c+TMC,

pcDNA/M2e-HSP70c without TMC, pcDNA/M2e and PBS, respectively, during light anesthesia with Ketamin/xylasine solution on days 0 and 21. The intranasal administered formulations were given through instillation in a total volume of 100µg/10µL (5µL into each nostril) with a micropipette tip, which is an appropriate volume to prevent variable bio-availability due to the deposition of the formulation in the lung and lower respiratory tract (Amidi et al., 2007).

Serological evaluations: Serum samples were taken prior to the first immunization, 2 weeks after the first immunization, and 2 weeks after the second immunization to study M2e-specific antibody responses. The ELISA assay was performed as described previously (Amidi et al., 2007) with some modifications. Briefly, 96 well plates (Maxisorb, Nunc, Denmark) were coated with 100µL of synthetic M2e-peptide solution (1µg/mL) in 50mM sodium bicarbonate buffer, pH 9.6, and incubated overnight at 4 C°. The plates were washed with phosphate buffered saline (PBS) and blocked with 3% milk in PBS containing 0.05% Tween-20 (milk-PBST). The testing samples were diluted in PBST and loaded on the peptide coated plates. The plates were incubated with different serum samples and then incubated with 1:10000 goat antimouse IgG-HRP conjugates (Sigma-Aldrich) for 1 hr at 37°C. The color reaction was developed with 3, 3', 5, 5'-tetramethylbenzidine (TMB) at OD 450 nm. In order to compare the groups, the average of A450 values of different sera was analyzed.

Statistical analysis: Statistically significant differences amongst M2e antibody titres of different sera were analyzed using the SPSS 13.0 software (SPSS Inc., Chicago, USA). Data were analyzed for their significance ($p<0.05$) by the one-way analysis of variance when the variance between the groups was homogeneous and the distribution of the data was normal. When the normality test or the homogeneity of the variance test failed the nonparametric test of Kruskal-Wallis was used.

Results

Characterization of the pcDNA/M2e-HSP70c-loaded TMC particles: The pcDNA/M2e-HSP70c+TMC particles had an average diameter of 102±27nm with a polydispersity index of 0.05±0.02 (Figure 1). The zeta-potential of the antigen loaded particles was +32±1mV and the loading efficacy was 91.2%. (Data not shown)

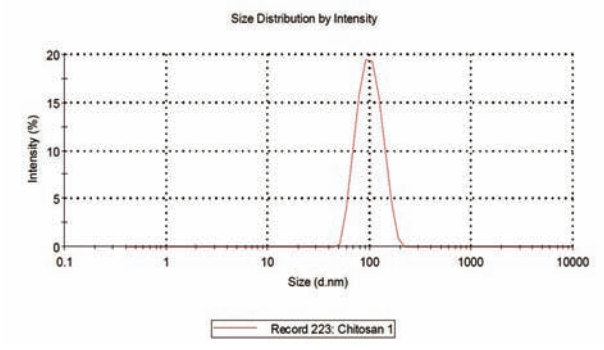

Figure 1. The particle size of chitosan-DNA is measured on a Zetasizer® 3000 (Malvern Instruments, UK). The pcDNA/M2e-HSP70c+TMC particles has an average diameter of 102±27nm with a polydispersity index of 0.05±0.02.

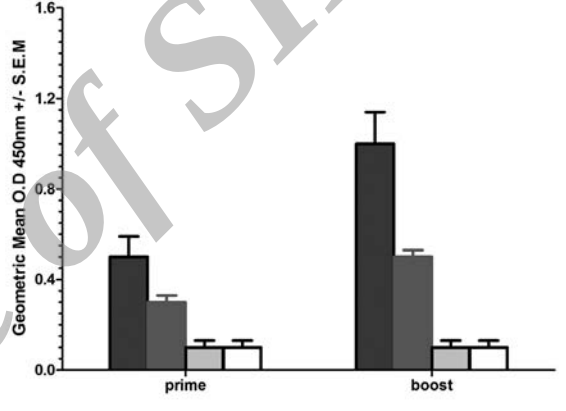

Figure 2. M2e-specific antibodies were determined by ELISA. Anti-sera diluted of 1/100 were collected from individual mice immunized with pcDNA/M2e-HSP70c+TMC ( ), pcDNA/M2e-HSP70c without TMC ( ), and pcDNA/M2e ( ), at 3 PBS ( ), weeks after the first immunization (1st immunization) and 2 weeks after the second immunization (2nd immunization), separately. Each bar represents the geometric mean antibody responses for each group of immunized mice with the standard error (Geometric Mean±SEM). pcDNA/M2e-HSP70c+TMC administrated group shows higher M2e specific IgG compared with other groups ($p<0.05$, one-way ANOVA).

Systemic antibody responses in i.n. immunized mice: To investigate the suitability of the antigen-loaded TMC particles for i.n. vaccination, the serum responses of mice were compared after a single i.n. vaccination with antigen alone and antigen-loaded

TMC particles. After a single i.n., immunization free pcDNA/M2e-HSP70c and pcDNA/M2e were poorly immunogenic, showing undetectable serum IgG titers in some of the vaccinated animals (Figure 2). In contrast, the pcDNA/M2e-HSP70c+TMC particles were able to significantly generate higher ($p<0.05$) IgG antibody titers in all mice (Figure 2). These results point to a strong immunostimulating effect of the TMC particles upon i.n. administration. Since the i.n. vaccination usually requires booster immunizations in order to induce strong immune responses, the effect of i.n. booster vaccinations on the systemic antibody response was studied. As shown in Figure 2, even after two booster immunizations, i.n. administered free antigen induced weak systemic immune responses while the second booster in the antigen-TMC group induced a further increase in the total IgG immune response (Figure 2).

Discussion

The M2e domain has been found to be highly conserved in most influenza A strains (Ito et al., 1991, Liu et al., 2005), making it an attractive target for a universal vaccine.

Different studies have shown the adjuvant properties of HSP70. Haug et al., (2005) showed that HSP70 can enhance the proliferation of human CD4+ T cells. HSP70 induces the production of proinflammatory cytokines by the monocyte-macrophage system and it, in turn, leads to the activation of innate immunity (Tsan et al., 2007). The immunomodulatory function of HSP70 is based on its interaction with the receptors present on the professional APCs (Ebrahimi et al., 2010).

We have previously shown that the M2e-HSP70c plasmid (pcDNA/M2e-HSP70c) when injected subcutaneously in mice by the In vivo electroporation can induce the humoral immune response against the Influenza virus (Ebrahimi et al., 2012).

Mucosal immune responses provide a first line of defense against infectious agents that use mucosal surfaces as a portal site of entry.

The nasal mucosa and pulmonary epithelium provide a large surface for the absorption of recombinant DNA and proteins (Amidi et al., 2006).

Proper delivery systems for the nasal administration should protect antigens against degradation, prevent their rapid elimination from the nasal cavity, and enhance their absorption and/or uptake across epithelial barriers.

The present study intended to obtain these properties through the encapsulation of pcDNA/ M2e-HSP70c into N-trimethyl chitosan (TMC).

TMC is a derivative of chitosan. Chitosan and positively charged chitosan complexes will bind strongly to negatively charged sites in the mucin. This leads to prolonged antigens residence in the nasal cavity with a half-life of 45 min as compared to about 15 min for control solutions (Soane et al., 1999).

Thus, chitosan can overcome the mucociliary function in the nasal cavity, protect an antigen from the enzymatic degradation, and provide the efficient delivery of antigens to mucosal-associated lymphoid tissue (MALT) and antigen presenting cells (APC) lined between the respiratory epithelial cells (Bienenstock et al., 2005).

Over the past few years, various groups have worked on the chitosan as an alternative non-viral gene delivery system for the delivery of therapeutic genes as well as gene vaccines (MacLaughlin et al., 1998; Koping-Hoggard et al., 2001; Mao et al., 2001; Ishii et al., 2001). To date, most of the studies have been undertaken with reporter genes and through routes other than the nasal (MacLaughlin et al., 1998; Mao et al., 2001; Ishii et al., 2001; Borchard et al., 2001). However, there are few studies have been also conducted about the use of chitosan complexes to deliver DNA vaccines especially through the nasal route. Roy et al. (1999) evaluated a chitosan based oral DNA vaccine system for vaccination against the peanut allergy in a mouse model, and obtained good protection.

In this study, i.n. administration of the encapsulated pcDNA/M2e-HSP70c into the TMC particles significantly enhanced the systemic immune response, compared to free pcDNA/M2e-HSP70c+TMC and pcDNA/M2e. In comparison to the free antigen, the prolonged residence time of the encapsulated antigen in the nasal cavity can provide enough time for M cells as well as epithelial cells to uptake the plasmid. The present study showed that the encapsulation of M2e/HSP70c into the N-trimethyl chitosan (TMC) could induce higher humoral immune response against M2e. Thus, TMC is a promising vehicle for the nasal delivery of influenza anti-

gens and, most likely, other antigens.

Acknowledgments

This work was supported by Tehran university grant for project No. 7502015/6/17.

References

1. Almeida, A.J., Alpar, H.O., Brown, M.R.W. (1993) Immune response to nasal delivery of antigenically intact tetanus toxoid associated with poly (l-lactic acid) microspheres in rats, rabbits and guineapigs. J Pharm Pharmacol. 45:198-203.
2. Alpar, H.O., Almeida, A.J., Brown, M.R.W. (1994) Microsphere absorption by the nasal mucosa of the rat. J Drug Target. 2: 147-9.
3. Alpar, H.O., Somavarapu, S., Atuah K.N., Bramwell, V.W. (2005) Biodegradable mucoad-hesive particulates for nasal and pulmonary antigen and DNA delivery. Drug Deliv Rev. 57: 411-430.
4. Amidi, M., Romeijn, S.G., Verhoef, J.C., Junginger, H.E., Bungener, L., Huckriede, A., et al. (2007) N-Trimethyl chitosan (TMC) nanoparticles loaded with influenza subunit antigen for intranasal vaccination: Biological properties and immunogenicity in a mouse model. Vaccine. 25: 144-153.
5. Amidi, M., Mastrobattista, E., Jiskoot, W., Hennink, W.E. (2010) Chitosan-based delivery systems for protein therapeutics and antigens. Adv Drug Deliv Rev. 62:59-82.
6. Amidi, M., Romeijn, S.G., Borchard, G., Junginger, H.E., Hennink, W.E., Jiskoot, W. (2006) Preparation and characterization of protein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system. J Control Release. 111: 107-16.
7. Bienenstock, J., McDermott, M.R. (2005) Bronchus and nasal-associated lymphoid tissues. Immunol Rev. 206: 22-31.
8. Borchard, G. (2001) Chitosans for gene delivery. Adv Drug Deliv Rev. 5: 145-50.
9. Davis, S.S. (2001) Nasal vaccines. Adv Drug Deliv Rev. 51: 21-42.
10. de Haan, L., Haan, L., Verweij, W.R., Holtrop, M., Brands, R., van Scharrenburg, G.J., et al. (2001) Nasal or intramuscular immunization of mice with influenza subunit antigen and the B subunit of *Escherichia coli* heat-labile toxin induces IgA- or IgG-mediated protective mucosal immunity. Vaccine. 19: 2898-907.
11. De Filette, M., Min Jou, W., Birkett, A., Lyons, K., Schultz, B., Tonkyro, A., et al. (2005) Universal influenza A vaccine: optimization of M2-based constructs. Virology. 337: 149-61.
12. Ebrahimi, S.M., Tebianian, M. (2011) Role of Mycobacterial Heat Shock Protein 70 (mHSP70) as Genetic Vaccine Adjuvants. World Appl Sci J. 14: 1569-1575.
13. Gaston, J.S.H. (2002) Heat shock proteins and innate immunity. Clin Exp Immunol. 127: 1-3.
14. Grandea, R.A.G., Olsen, O.A., Cox, C.T, Renshaw, M., Hammond, P.W., Chan-Hui, P.Y., et al. (2010) Human antibodies reveal a protective epitope that is highly conserved among human and nonhuman influenza A viruses. Proc Natl Acad Sci USA. 107: 12658-63.
15. Haug, M., Dannecker, L., Schepp, C.P., Kwok, W.W., Wernet, D., Buckner, J.H., et al. (2005) The heat shock protein HSP70 enhances antigen-specific proliferation of human CD4+ memory T cells. Eur J Immunol. 35: 3163-72.
16. Hay, A.J., Gregory, V., Douglas, A.R., Lin, Y.P. (2001) The evolution of human influenza viruses. Philos Trans Roy Soc Lond B: Biol Sci. 356: 1861-70.
17. Holmgren, J., Czerkinsky, C. (2005) Mucosal immunity and vaccines. Nat Med. 11: 45-53.
18. Illum, L. (1998) Chitosan and its use as a pharmaceutical excipient. Pharm Res. 15: 1326-31.
19. Ishii, T., Sato, T., Okahata, Y. (2001) Mechanism of cell transfection with plasmid/chitosan complexes. Biochim Biophys Acta. 1514: 51-64.
20. Ito, T., Gorman, O.T., Kawaoka, Y., Bean, W.J., Webster, R.G. (1991) Evolutionary analysis of the influenza A virus M gene with comparison of the M1 and M2 proteins. J Virol. 65: 5491-8.
21. Johnson, N.P., Mueller, J. (2002) Updating the accounts: global mortality of the 1918- 1920 "Spanish" influenza pandemic. Bull Hist Med. 6: 105- 115.
22. Koping-Hoggard, M., Tubulekas, I., Guan, H., Edwards, K., Nilsson, M., Vårum, K.M., et al. (2001) Chitosan as a nonviral gene delivery system. Structure-property relationships and characteristics compared with polyethylenimine in vitro and after lung administration in vivo. Gene Ther. 8: 1108-21.

23. Liu, W., Zou, P., Ding, J., Lu, Y., Chen, Y.H. (2005) Sequence comparison between the extracellular domain of M2 protein human and avian influenza A virus provides new information for bivalent influenza vaccine design. Microbes Infect. 7: 171-7.
24. Lowrie, D.B., Tascon, R.E., Bonato, V.L., Lima, V.M., Faccioli, L.H., Stavropoulos, E., et al. (1994) Therapy of tuberculosis in mice by DNA vaccination. Nature. 400: 269-71.
25. MacLaughlin, F.C., Mumper, R.J., Wang, J., Tagliaferri, J.M., Gill, I., Hinchcliffe, M., et al. (1998) Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. J Control Rel. 56: 259-72.
26. Mao, H.-Q., Roy, K., Troung-Le, V.L., Janes, K.A., Lin, K.Y., Wang, Y., et al. (2001). Chitosan-DNA nanoparticles as gene carriers: synthesism, characterisation and transfection efficiency. J Control Rel. 70: 399-421.
27. Massa, C., Melani, C., Colombo, M.P. (2005) Chaperon and Adjuvant Activity of HSP70: Different natural killer requirement for cross-priming of chaperoned and bystander antigens. Cancer Res. 65: 7942-9.
28. Neirynck, S., Deroo, T., Saelens, X., Vanlandschoot, P., Jou, W.M., Fiers, W. (1999) A universal influenza A vaccine based on the extracellular domain of the M2 protein. Nat Med. 5: 1157-63.
29. Pawar, D., Goyal, A.K., Mangal, S., Mishra, N., Vaidya, B., Tiwari, S., et al. (2010) Evaluation of mucoadhesive PLGA microparticles for nasal immunization. AAPS J. 12: 130-7.
30. Poland, G.A., Jacobson, R.M., Ptargonski, P.V. (2007) Ptargonski Avian and pandemic influenza: an overview. Vaccine. 25: 3057-3061.
31. Qazi, K.R., Wikman, M., Vasconcelos, N.M., Berzins, K., Ståhl, S., Fernández, C. (2005) Enhancement of DNA vaccine potency by linkage of Plasmodium falciparum malarial antigen gene fused with a fragment of HSP70 gene. Vaccine. 23: 1114-25.
32. Rafati, S., Bolhassani, A. (2008) Heat-shock proteins as powerful weapons in vaccine development. Expert Rev Vaccines. 8: 1185-99.
33. Roy, K., Mao, H.Q., Huang, S.K., Leong, K.W. (1999) Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine peanut allergy model. Nat Med. 5: 387-91.
34. Soane, R.J., Frier, M., Perkins, A.C., Jones, N.S., Davis, S.S., Illum, L. (1999) Evaluation of the clearance characteristics of bioadhesive systems in human. Int J Pharm. 178: 55-65.
35. Soane, R.J., Hinchcliffe, M., Davis, S.S., Illum, L. (2001) Clearance characteristics of chitosan based formulation in the sheep nasal cavity. Int J Pharm. 217: 183-91.
36. Suzue, K., Young, R.A. (1996) Adjuvant-free HSP70 fusion protein system elicits humoral and cellular immune responses to HIV-1 p24. J. Immunol. 156: 873-9.
37. Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Bridges, C.B., Cox, N.J., et al. (2004) Influenza-associated hospitalizations in the United States. JAMA. 292: 1333-1340.
38. Turley, C.B., Rupp, R.E., Johnson, C., Taylor, D.N., Wolfson, J., Tussey, L., et al. (2011) Safety and immunogenicity of a recombinant M2e-flagellin influenza vaccine (STF2.4xM2e) in healthy adults. Vaccine. 29: 5145-52.
39. Verweij, W.R., D.H.L., Holtrop, M., Agsteribbe, E., Brands, R., Van, E.A., Scharrenburg, G.J. (1998) Mucosal immunoadjuvant activity of recombinant Escherichia coli heat-labile enterotoxin and its B subunit: induction of systemic IgG and secretory IgA responses in mice by intranasal immunization with influenza virus surface antigen. Vaccine. 16: 2069-76.
40. Vila, A., Sánchez, A., Janes, K., Behrens, I., Kissel, T., Jato, J.L.V., et al. (2004) Low molecular weight chitosan nanoparticles as new carriers for nasal vaccine delivery in mice. Eur. J. Pharm. Biopharm. 57: 123-131.
41. Zabeh Jazi, M.H., Dabaghian, M., Tebianian, M., Gharagozlou, M.J., najminezhad H., Ebrahimi, S.M. (2012) In vivo electroporation enhances immunogenicity and protection against influenza A virus challenge of an M2e-HSP70c DNA vaccine. Virus Res. 167: 219-25.
42. Zebedee, S.l., Lamb, R.A. (1998) Influenza A virus M2 protein: monoclonal antibody restriction of virus growth and detection of M2 in virions. J Virol. 62: 2762-72.

مجله طب دامی ایران، ۱۳۹۲، دوره ۷، شماره ۲، ۱۲۸-۱۲۳

استفاده از تری متیل کایتوزان در تجویز داخل بینی DNA کدکننده M2e-HSP70c در مدل موشی

مهران دباغیان[۱] سید محمود ابراهیمی[۲] غلامرضا نیکبخت[*۱] مجید تبیانیان[۳] علی رضایی مکرم[۳] مریم ایمان[۴] علیرضا توانگر رنجبر[۵]

۱) گروه میکروبیولوژی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران
۲) مرکز تحقیقات واکسن و ویروس، دانشگاه علوم پزشکی بقیه ا...، تهران، ایران
۳) بخش بیوتکنولوژی، موسسه تحقیقات واکسن وسرم سازی رازی، کرج، ایران
۴) مرکز تحقیقات آسیب های شیمیایی، دانشگاه علوم پزشکی بقیه ا...، تهران، ایران
۵) بخش ویروس شناسی، دانشگاه علوم پزشکی تهران، تهران، ایران

(دریافت مقاله: ۹ اسفند ماه ۱۳۹۱، پذیرش نهایی: ۳۱ اردیبهشت ماه ۱۳۹۲)

چکیده

زمینه مطالعه: شیوع آنفلوانزا در سطح جهانی خطری بالقوه برای بهداشت عمومی در دنیا است. واکسن های مخاطی قادرند سیستم ایمنی عمومی و مخاطی را تحریک کرده و موجب تولید آنتی بادی های IgG و IgA در مخاط شوند، و به این ترتیب دو لایه محافظت در برابر عوامل عفونی نظیر ویروس آنفلوانزا ایجاد می شود. سطح مخاطات پوشیده از موکوس می باشد که به طور پیوسته طی مکانیسمی به نام پاکسازی مخاطی مژه‌ای از سطح مخاط پاک شده و لایه موکوس جدید جایگزین می گردد. بدین ترتیب آنتی ژن تجویز شده از طریق داخل بینی فرصت کمی برای نفوذ به مخاط و تحریک سیستم ایمنی را دارد. **هدف:** در این مطالعه اثر تری متیل کایتوزان به عنوان حامل موثر برای DNA کدکننده M2e/HSP70c در تجویز داخل بینی در موش مورد بررسی قرار گرفت. **روش کار:** ژن M2e حراست شده ویروس آنفلوانزا که قادر به ایجاد ایمنی متقاطع در بین گونه های مختلف می باشد، در وکتور pcDNA 3.1 با قسمت c-terminal HSP70 متصل شده و پس از کپسوله شدن با یکی از مشتقات کایتوزان به نام تری متیل کایتوزان از راه داخل بینی تجویز شد. پس از کپسوله شدن پلاسمید ویژگی های فیزیکی ذرات حاصل توسط دستگاه Zetasizer® 3000 (Malvern Instruments, UK) بررسی شدند. **نتایج:** ذرات تولیدی دارای اندازه حدود ۹۰-۱۲۰nm بوده و دارای بار سطحی مثبت بودند. تجویز پلاسمید M2e/HSP70c به صورت کپسوله با TMC موجب تولید آنتی بادی IgG در گردش علیه M2e شد که میزان آنتی بادی تولیدی تفاوتی معنادار نسبت به تجویز M2e/HSP70c بدون TMC و pcDNA/M2e داشت. **نتیجه گیری نهایی:** در این تحقیق نشان داده شد که کپسوله کردن M2e/HSP70c در TMC موجب تقویت تحریک سیستم ایمنی هومورال بر علیه M2e/HSP70c شده بدون آنکه تأثیر منفی بر خاصیت کمک ایمن HSP70c داشته باشد.

واژه های کلیدی: آنفلوانزا، M2e، پروتئین شوک حرارتی ۷۰، تری متیل کایتوزان، تجویز داخل بینی

*)نویسنده مسؤول: تلفن: ۶۱۱۱۷۰۵۷(۲۱)۹۸+ نمابر: ۶۶۴۲۷۵۱۷(۲۱)۹۸+ Email: nikbakht@ut.ac.ir