P-041

Development of an oral vaccine against rotavirus

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INTRODUCTION

Rotaviruses are recognized as the major cause of diarrhoea in children worldwide leading to approximately half million deaths each year (Ciarlet 2009). Because of the significant morbidity and mortality associated with rotavirus diarrhoea, there is a need to develop a safe and effective vaccine. Immunization with plasmid DNA (pDNA) has recently been introduced as a new and effective strategy of vaccination. However, oral administration of a naked pDNA is highly susceptible to nuclease degradation. On the other hand, parenteral administration of vaccines was not found to be effective in inducing immunity at mucosal surface (Mestecky 1996). Administration of pDNA through oral route requires the use of an efficient drug delivery system. Chitosan nanoparticles have received considerable attention for delivering therapeutic peptides, protein and gene (Jayakumar 2010). The aim of this study was to prepare an oral vaccine for rota virus based on association of plasmid DNA with chitosan nanoparticles. Further objectives were to study the effect of formulation parameters on the % DNA loading and to evaluate the prepared vaccine in vivo using BALB/c mice. In this study VP7 DNA was used as rotavirus vaccine.

MATERIALS AND METHODS

Preparation of DNA vaccine Epizootic diarrhea of infant mice murine rotavirus EW outer capsid glycoprotein VP7 gene has been acquired from researchers working with the virus at Kind saud University (GenBank accession number U08430.1 GI:475696). Plasmid Bluescript (Stratagene), a derivative of plasmid pBR322 and phages M13 has been used as a carrier for the virus VP7. VP7 has been inserted into Bam H1 fragment of plasmid Bluescript SK. The constructed plasmid was extracted as described by (Ausubel 2002).

Preparation of chitosan nanoparticles Chitosan nanoparticles were prepared according to the method reported by (Akbuga 2004) with modifications to produce nanoparticles. The effect of formulation parameters on the DNA loading was investigated. These parameters include: type and concentration of cross-linking agent and polymer molecular weight.

Loading of plasmid – DNA Plasmid – DNA dispersed in phosphate buffer pH 7.4 containing chitosan nanoparticles were kept at 37 °C for 4 hours under shaking. After incubation, the suspension was centrifuged to remove the unloaded plasmid.

Determination of the adsorption efficiency of pDNA Adsorption efficiency of plasmid DNA in chitosan nanoparticles was determined by quantifying unloaded plasmid in the supernatant with the picogreen reagent. The loading content was calculated according to the following equation: (%) DNA adsorbed = (1 – free plasmid / Total amount of plasmid) x 100

Morphology observation The morphology characteristics, shape and size of chitosan nanoparticles were studied using scanning electron microscopy.

In vivo studies The selected formulations of nanoparticles were inoculated orally into adult BALB/c mice by proximal esophageal intubation with a dose equivalent to 75µg DNA. Mice were sacrificed at day 28 post inoculation and blood was collected for analysis. Enzyme-linked immunosorbent assays (ELIZA) were performed on blood samples for detection of IgG, IgM, and IgA antibodies.

RESULTS AND DISCUSSION

Preparation method used yielded stable, reproducible and spherical nanoparticles. Scanning electron micrograph shows that chitosan nanoparticles are spherical and in the size range between 200nm to 500 nm.

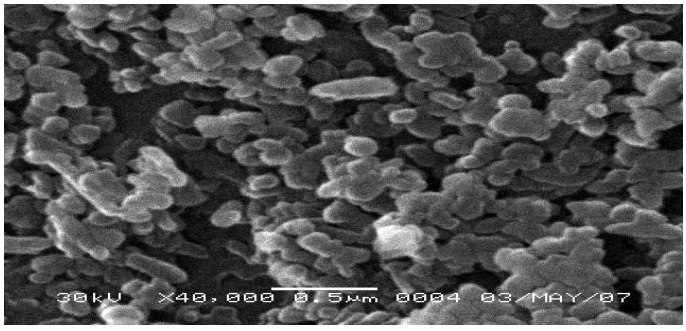

Figure 1: SE micrograph of chitosan nanoparticles.

The effect of experimental conditions and additives used in formulations on the adsorption efficiency of DNA were studied and the results are presented in table 1. The table shows that the use of gluteraldehyde crosslinking agent reduced the adsorption efficiency of DNA. In addition, the content of DNA in nanoparticles decreased as the concentration of gluteraldehyde increased. Addition of TPP to formulations has slightly increased the adsorption efficiency of DNA in comparison with non crosslinked particles. Particles containing TPP exhibited

adsorption efficiency higher than 90%. However, increasing the concentration of TPP in the particles didn't have a significant influence on the percentage of DNA adsorbed. The effect of the molecular weight of the polymer of the adsorption efficiency of DNA onto the surface of chitosan naoparticles is presented in table 1.

Low and high molecular weight chitosan exhibited an adsorption efficiency values higher than 95%. On the other hand, chitosan particles prepared using medium molecular weight possessed lower DNA loading.

Table 1: Effect of formulation parameters on % DNA loading of nanoparticles.

Conc. of gluteraldehyde (%)	pDNA adsorbed (%) ± SD	Conc. of TPP (gm)	pDNA adsorb (%) ± SD	Mol. weight of the polymer (%)	pDNA adsorb (%) ± SD
Original particles	87.259 ± 0.490	Original particle	87.259 ± 0.490	Low	96.034± 1.038
5	67.77 ± 5.515	0.01	93.512 ± 2.433	Medium	87.259 ± 0.490
10	40.84 ± 8.739	0.02	90.825 ± 1.146	High	95.817± 0.1726
15	34.608 ± 5.103	0.03	92.006 ± 1.297		

Animal studies performed yielded the following results as depicted in figure 2:

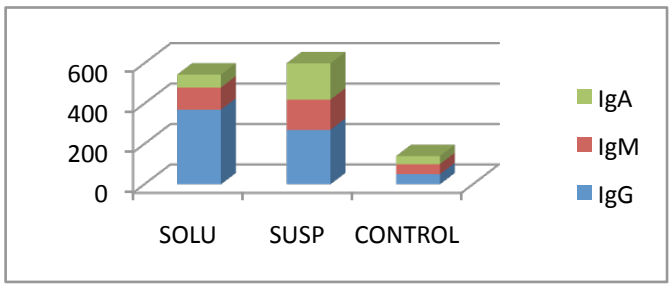

Figure 2: Mean antibody titres for the 3 groups of animals.

Mean titer of antibodies detected by ELISA in sera from the 3 groups of animals: those given naked plasmid in solution (SOLU), those given plasmid in nanoparticles (SUSP) and the control group.

From these results it was apparent that both naked plasmid and plasmid in nanoparticles yielded a statistically significant higher antibody response for the three antibody classes, namely IgG, IgM and IgA than the control group ($p \leq 0.05$).

However, comparing naked plasmid versus encapsulated plasmid showed that IgG was higher in the group given naked plasmid but this was not significant ($p \geq 0.05$). This could be because two of the mice in the second group were low responders. It has been reported that serum IgG titers after oral immunization were significantly lower ($p<0.05$) than IgG titers after intramuscular immunization and the IgG titers after oral administration of particles was lower than formaldehyde inactivated rhesus rotavirus (Sturesson 2000). As the current study involved one point sampling at 28 days, a slower release from the nanoparticles could have contributed in the lower IgG titer observed in the suspension group, the sample being taken at an early stage in the release of antigen from the nanoparticles with more antigen being released later in time. As the release pattern results in different ways of delaying, prolonging or otherwise altering rotavirus processing and presentation by APC which in turn causes differences in the immunological response. Therefore, a second dose might have been required to overcome the low transfectioon rate and produce a more favorable IgG antibody response. Loss of antigenicity during nanoparticles formulation has been ruled out by using suitable buffers and no solvents. On the other hand IgM and IgA were produced in statistically higher titres in the encapsulated plasmid group ($p \leq 0.05$). As IgM antibodies upon primary immunization appear earlier than IgG antibodies, the causes of lower IgG antibody response in the nanoparticle group versus the naked DNA group do not apply here. The mechanisms responsible for protection against rotavirus are not completely understood, although it has been shown that secretory IgA antibodies play a major role. Hence, the high level of IgA antibodies after oral administration of the oral encapsulated nanoparticles indicates that this system might be suitable as an oral rotavirus vaccine.

CONCLUSION

The developed vaccine seems promising but further testing of the formulated vaccine is warranted to address the stated concerns together with studies to show the impact of such a vaccine when administered simultaneously with other oral vaccines such as polio vaccine.

REFERENCES

- Ciarlet et al. (2009) Development of a rotavirus vaccine: Clinical safety, immunogenicity, and efficacy of the pentavalent rotavirus vaccine, RotaTeq. Vaccine (27S) 71-82.
- Mestecky et al. (1996) Current options for vaccine delivery systems by mucosal routes. J Control Rel. (48) 243-257.
- Jayakumar et al (2010) Chitosan conjugated DNA nanoparticles in gene therapy.Carbohydrate polymers. (79) 1-8.
- Ausubel et al. (2002)Short propocol in molecular biology. John Wiley and sons, (5th Ed.) Inc, USA.
- Akbuga et al. (2004). Plasmid-DNA loaded chitosan microspheres for in vitro IL-2 expression. Europ J Pharm and Biopharm, (58) 501-507.
- Sturesson et al. (2000). Comparison of poly (acryl starch) and poly(lactide-co-glycolide) microspheres as drug delivery systems for a rotavirus vaccine. J Control Rel. (68) 441-450.