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Study on Cytotoxicity and Virus Titration of Chitosan Nanoparticles Encapsulated Live PPR Virus Vaccine

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A B S T R A C T

The cytotoxicity and virus titration of chitosan nanoparticles encapsulated live PPR virus vaccine (PPRV-CS-NPs) was analyzed in this study. Live attenuated PPR virus vaccine (AR-87) was encapsulated in chitosan nanoparticles using ionic gelation technique. The PPRV-CS-NPs were tested for cytotoxicity study in Vero cells with different concentrations (1-40µg).The cell viability was more than 80 per cent with all the concentration of the PPRV-CS-NPs tested. This indicates that the PPRV-CS-NPs are not toxic even in concentration higher than the required vaccine dose. The viability of PPRV (AR-87) in the PPRV-CS-NPs were tested by releasing the virus from nanoparticles by trypsin digestion. The virus titration was carried out in Vero cells and the titre was found to be $10^{4.6}$ $TCID_{50}$/ml indicating that chitosan encapsulation was able to maintain virus infectivity.

Introduction

Peste des petits ruminants (PPR) or goat plague is a highly contagious viral disease of small ruminants such as sheep and goat (Dhar *et al*., 2002; Asim *et al*., 2009). Peste des petits ruminants virus is transmitted between animals such as sheep and goat and other small ruminants (Furley *et al*., 1987) through inhalation of aerosols and direct contact with ocular / nasal secretions, faeces, contaminated water and feed troughs (Saliki *et al*., 1993).

In India the first outbreak of PPR was reported by Shaila *et al*., (1989) and the disease has become endemic in India and causing severe economic losses to the small ruminant population of the country. The disease is controlled in endemic areas through administration of a live attenuated PPRV vaccine (Diallo, 2003). Three live attenuated vaccines currently available for use in India include: Sungri 96, Arasur 87 and Coimbatore 97 (Saravanan *et al*., 2010). The live attenuated PPR vaccines are thermolabile and require cold chain maintenance till administration.

In recent years attention has been directed towards the use of nanoparticles as delivery vehicles for vaccines. Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. The vaccine antigen is either encapsulated within or decorated onto the

surface of the nanoparticles. As a polyatomic, non- atomic, biodegradeable and biocompatible polymer, chitosan has attracted significant attention and can encapsulate a range of bioactive agents including proteins and peptides.

Chitosan nanoparticles can be prepared using various formulation methods to release active ingredients (such as proteins, peptides and DNA vaccines) in a sustained manner over a prolonged period. Among the encapsulation methods ionic cross linking method has received significant attention in recent years because the processes used are simple and mild for protein and viruses.

Materials and Methods

Preparation of chitosan nanoparticles

Vero cells adapted PPR vaccine virus (AR-87) maintained in the Department of Veterinary Microbiology, Madras Veterinary College, Chennai -7 was used in the study. Chiosan solution was prepared by dissolving chitosan (1mg/ml and 1.5 mg/ml) with sonication in an aqueous solution of 4% acetic acid. Sodium Tripolyphosphate (TPP) was dissolved in triple distilled water at the concentration of 1mg/ml and 1.5mg/ml. PPRV-CS-NPs was prepared using ionic cross linking method (Akalya *et al*., 2017).

In vitro cytotoxicity of PPRV-CS-NPs

Vero cells were transferred to 96 well plates at 100µl per well and cultured at 37°C in 5 per cent CO_2 incubator. PPRV-CS-NPs were diluted to contain 1, 10, 20, 30 and 40µg/200µl and added to vero cells. The 96 well plates were incubated in 5 per cent CO_2 incubator at 37°C for 24-72 hr. Each concentration was added in duplicates/triplicates. Control well contained cells along with Dulbecco's Minimum Essential Medium (DMEM) and Fetal Bovine Serum (FBS). MTT dye (5 mg/ml) was dissolved in triple distilled water. At the end of exposure period the nanoparticle suspension was removed and 200µl of fresh DMEM and 50µl of MTT dye were added to all wells. The plates were wrapped in alumininum foil and kept at 37°C for 4 hrs in CO_2 incubator. The medium containing dye was removed and the formazan crystals were dissolved by adding 200µl of DMSO. The absorbance was recorded at 570nm and the per cent of cell viability was calculated with the following formula,

Cell viability (%) = At/Ac X 100

Where, At - Absorbance of test
Ac - Absorbance of control

Determination of viral titre in the PPRV-CS-NPs

One hundred micrograms of dried PPRV-CS-NPs were added in to 10 ml of PBS buffer (pH 7.2). Subsequently 2 ml of trypsin was added to the above solution and then digested for 72 hr at 4°C with a concentration of trypsin solution at 0.25% and centrifuged for 5 min at 1200g. The obtained supernatent was diluted with sterile saline by a serial of 10 fold dilution and 10^{-1} and 10^{-8} were inoculated into the vero cells and incubated at 37°C in 5 per cent CO_2 incubator. Each dilution was tested in five replicates along with controls. The plates were incubated at 37°C in 5 per cent CO_2 incubator and were examined for the development of CPE. Fifty per cent end point was calculated as per the method of Reed and Muench (1938).

Results and Discussion

Cytotoxicity study was performed to assess the cell viability after exposure to the PPRV-CS-NPs. Vero cells were exposed to different

concentrations of PPRV-CS-NPs from 1µg to 40 µg and the cell viability was found to be more than 80 per cent in the concentration tested (Table 1). Though for vaccination of the animal less than 10µg of PPRV-CS-NPs would be needed the nanoparticles were not toxic even at 40 µg concentration which indicated that the biocompatibility and the safety of the nanoparticles. The obtained results were in agreement with chitosan loaded with ciprofloxacin which was performed in human fibroblasts and found that there was no difference between the cells cultured in the medium containing chitosan nanoparticles and the cells cultured in the control medium (Hui and Changyou Gao, 2008).

Table.1 *In vitro* cytotoxicity study of PPRV - CS - NPs by MTT Assay

S.NO	Concentration of PPRV-CS-NPs	Absorbance value	(Absorbance value of test /Absorbance value of control) X 100
	Control (0µg)	1.819	100%
1.	1µg	1.697	93.2%
2.	10µg	1.527	83.9%
3.	20µg	1.5125	83.1%
4.	30µg	1.468	80.7%
5.	40µg	1.463	80.4%
6.	50µg	1.385	76.1%

In the present study the infectivity titre of the PPRV-CS-NPs were found to be $10^{4.6}$ $TCID_{50}$/ml which is one log lesser than vaccine virus used for the preparation. The decrease in virus infectivity titre may be due to the prolonged trypsin digestion carried out for the release of virus from the PPRV-CS-NPs. The findings of this study indicate that chitosan encapsulation by ionic gelation technique is able to maintain the virus infectivity and thus chitosan nanoparticles can act as delivery vehicle for live attenuated PPR vaccine.

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