Capsular K-antigen-PLGA Nano conjugated Vaccine against *Klebsiella pneumoniae pneumoniae K201* Infection

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**Dear Editor in Chief**

*Klebsiella pneumoniae* is the most common pathogenic bacterium in the *Klebsiella* genus (1). Every year, about two million children under the age of 5 die from pneumonia. Due to the acquired and inherent resistance of isolated *Klebsiella pneumoniae* against a wide range of antibiotics, its control and treatment appear to be critical (2). Present study aimed to design a vaccine, containing Polyactic-co-glycolic acid (PLGA) nanoparticles and *Klebsiella pneumoniae* K201 capsule antigen. The capsule antigen was extracted by centrifugation, and the supernatant was dialyzed for filtration from HR S200 gel column; it was detoxified with phenol.

Then the capsular antigen was conjugated to PLGA nanoparticles by W/O/W method. Infrared spectroscopy (FT-IR) and atomic force microscopy (AFM) was used to confirm nanoparticles conjugation. Limulus amoebocyte lysate assay (LAL) was also performed to evaluate the endotoxin of the designed vaccine (3). In this study, 10 mice were used to assess the non-toxicity of the designed vaccine candidate. Each of BALB/C mice was injected intramuscularly with 10 μg of vaccine, containing capsule antigen in association with PLGA nanoparticles and Methoxy polyethylene Glycol Polycaprolactone (MPEG-PCL) without capsule antigen, separately.

One week later, the mortality rate in mouse groups were evaluated and the immunogenicity results were analyzed by one-way analysis of variance. SPSS software version 18 was used for statistical analysis (4,5). To determine pyrogenicity of the vaccine candidates, the rabbits were selected in groups of three, and the body temperature was examined by putting a thermometer in the rectum, every 15 minutes for one hour (6,7). Also, through the marginal vein of the rabbit ear, the prepared sample was injected and the animal's body temperature was recorded every hour; this process continued for 3 hours.

The average temperature was calculated. The designated substance was considered non-pyrogenic injectable, due to a slight increase (0.4°C-0.6°C) in temperature of each rabbit; temperature change was less than 1.5°C in all three rabbits (8,9). In the present study, all experiments such as maintenance and feeding of animal patterns were performed using the book entitled ‘guide for the care and use of laboratory animals’ book; it is based on the NIH protocols (10).

The success of antigen and nanoparticles conjugation was based on the size and charge of antigen-containing nanoparticles; it was confirmed by the Zetaziser. In the FT-IR results, the shape of the corresponding peaks confirmed the presence of antigen-functional groups in the nanoparticle structure and the formation of bonds. AFM microscopic images of nanoparticles containing capsular antigen and nanoparticles before conjugation showed an increase in the binding sites of nanoparticles after conjugation. Change from initial sharpness to puffiness after conjugation proved the success of antigen transport by nanoparticles.

Fever was not observed in rabbits and mortality was checked in BALB/C mice. The results showed, that the vaccine could be recommended for animal studies with more samples or the first phase of the clinical trial studies. Investigation of mice serum by Elisa showed that antibody secretion was well done; in the next stage after becoming vaccinated with the

Ethical Considerations
Ethical issues (Including plagiarism, informed consent, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely considered by the authors.

Conflict of Interest
Authors declared no conflict of interest.

References


