Vaccine-coated Microcrystals: Enhanced Thermal Stability of Diphtheria Toxoid

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Introduction

Vaccine instability to heat is thought to lead to the wastage of half of all supplied vaccines worldwide [1-3].

The problems are especially acute in developing countries where adequate electrical power and refrigeration are often lacking; however improper storage have also been reported in temperate countries and in the developed world [2].

To overcome the problems associated with the cold-chain, different means of enhancing the thermal stability of vaccines have been explored, such as the formulation of dried vaccine formulations via lyophilisation, spray drying and spray freeze-drying [4-6]. The disadvantage with drying, however, is the fact that the drying process itself can change vaccine conformation and contribute to vaccine instability.

Other means of enhancing vaccine stability is the use of optimal pH, ionic strength, excipients such as, osmolytes (e.g. sugars and amino acids), proteins (e.g. albumin, gelatine), surfactants (e.g. Tweens), formaldehyde, chelators (e.g. EDTA), salts (e.g. magnesium chloride) [2] and the substitution of deuterium oxide for water [7].

In our laboratories, we are investigating novel protein-coated microcrystals (PCMC) to formulate a wide range of biomolecules including proteins, peptides, DNA, RNA [8]. This novel particle engineering approach has been shown to enhance protein stability [9]. It was hypothesised that the PCMC might be a means of delivering heat-labile vaccines.

Aim: The aim of this study was to investigate whether vaccine stability can be enhanced by formulating the vaccine immobilised on micro-crystals.

Methods

Preparation of vaccine-coated micro-crystals:

Appropriate volumes of the Diphtheria Toxoid (DT, a model antigen) solution and the crystalline-forming material (in this case L-glutamine) were added together. The mixture was then added into propan-2-ol (a water-miscible organic solvent). Dehydration of the vaccine and of the crystal-forming material resulted in co-precipitation of the two components, resulting in the formation of micro-crystals coated with vaccine. The micro-crystals were then dried.

Characterisation of vaccine-coated micro-crystals:
Scanning electron microscopy was conducted on the crystals to determine crystal size and morphology. The actual loading of DT in micro-crystals was determined by Bradford assay.

Investigation into vaccine stability to heat:
DT-coated crystals and the controls (free DT solution) were incubated at room temperature for 2 weeks, at 37^{0}C for 2 weeks or at 45^{0}C for 2 days, prior to suspension in a phosphate buffered saline and intramuscular administration to groups of female Balb/c mice (n=5). Each mouse received 5µg of DT in 50µL of suspension. Booster doses of the same formulations were administered 4 weeks later. The mice were bled from the tail vein on days 21 and 42 and the levels of antigen-specific antibody in the serum was determined using ELISA assays.

Results and Discussion

The vaccine-coated micro-crystals were formed when the vaccine and the crystalline-forming material, L-glutamine, co-precipitated out due to their dehydration, when a non-solvent was added to the aqueous medium. The crystals were then dried to produce a free-flowing powder.

Scanning electron microscopy of the crystals revealed flat plates, with an average size of between 5 and 10nm (figure 1). The actual loading of DT were calculated to be 3.95% w/w.

Antibody responses (primary and secondary) were similar when free and DT-coated microcrystals, incubated at 4 ^{0}C, at room temperature and at 37 ^{0}C for 2 weeks, were intramuscularly administered to mice (figures 2,3).

Free DT seems to be stable at these temperatures. This concords with the literature [2].

In contrast the antibody responses to free DT, which had been incubated at 45 ^{0}C for 2 days were severely reduced (figures 2, 3). This indicates that degradation of DT had occurred at this temperature.

☺ Antibody responses to DT immobilised onto micro-crystals, however, were not affected even when the formulation had been incubated at 45 ^{0}C for 2 days (figures 2.3). This indicates that coating DT onto micro-crystals seems to protect the antigen from heat degradation.

Fig. 2

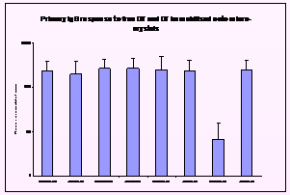
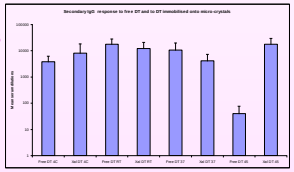


Fig. 3



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Conclusions

The study shows that immobilisation of DT enhanced its heat stability when the vaccine was incubated at 45^{0}C for 2 days.

The potential application of the PCMC technology as a means of formulating heat-labile vaccines into stable preparations has been shown.

References:

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