Design of a lipid nanovesicle system encasing bacteriophages for inhalational therapy: A proof of concept.

Abstract

Inflammatory diseases that occur in the pharynx and involving both the adenoids and tonsils are important not only for being very frequent, but also because they often require minor surgery for their resolution. These structures have immunological functions leading to production of antibodies, and work in the local immunity of the pharynx, and protection of the entire body. The most common etiologic agent of tonsillitis is *Streptococcus pyogenes*, an important pathogen responsible for many infections of the respiratory tract, since inhalation therapy is considered to be favorable to accelerating the action of bacterial predators. Additionally, a smaller amount of bioactive compound that is needed, thus preventing or reducing possible side effects. As a proof of concept, droplets are themselves dispersed in a continuous aqueous phase.

Experimental procedures

PRODUCTION OF MULTIPHASE BACTEROIDAL-SCALING LIQUID NANODELIVERY

Production of multiple emulsions encapsulating *Streptococcus* aerosols with T4- bacteriophage was carried out using an Ultra-Turrax (model T25D from IKA) under heating (ca 40°C). T4-bacteriophages were suspended in the (inner) aqueous phase (10 µL of bacterial suspension), as observed under an optical microscope at maximum resolution (Oil immersion, x1000).

The preliminary results obtained for the antimicrobial (lytic) properties of the nanoemulsions produced were evaluated and followed throughout a prolonged storage at room temperature. The results obtained are displayed in Figure 2 and 3.

Optimization of the formulation parameters

Microcalorimetric analysis was performed in a differential scanning calorimeter (Shimadzu, Kyoto, Japan). For every calorimetric assay, ca. 10 mg of emulsion were weighed (using a microbalance) and directly submitted to slit heating (under nitrogen); (v) inside the chamber, the deep-frozen sample was fractured, undergone sublimation (during 90 s to 300 s) by gently increasing the temperature from -140 °C to 35 °C, and was coated with Au/Pd (during 5× 30 s); finally, the sample was transferred into the SEM chamber for microscopy analysis.

OPTIMIZATION OF THE NANOFORMULATION

Several variables were studied, viz. lipid nature, poloxamer nature, size, Zeta concentration and Tween 60 concentration.

Table 1. Optimization of processing conditions leading to an optimal nanomunification *Teiichphage*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Activity (%)</th>
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<tbody>
<tr>
<td>Lipid nature</td>
<td>O/W, W/O/W</td>
<td>90</td>
</tr>
<tr>
<td>Poloxamer nature</td>
<td>Tween 80</td>
<td>95</td>
</tr>
<tr>
<td>Size</td>
<td>150 nm</td>
<td>97</td>
</tr>
<tr>
<td>Zeta concentration</td>
<td>0.5 mV</td>
<td>98</td>
</tr>
<tr>
<td>Tween 60 concentration</td>
<td>5%</td>
<td>99</td>
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The effect of simultaneously increasing the amounts of Tween 80 and lecithin were the highest in the increase of *Zeta Potential* (from more negative towards less negative values), presumably due to accumulation of absorbed ions at the particle surface. A certain concentration of Tween 80 proved to be suitable in producing lipid nanovesicles with dilute *Zeta Potential* and higher hydrodynamic sizes, throughout storage time.

Microscopic analysis of the nanoveccles

Lipid nanovesicles were analyzed by differential scanning calorimetry (Shimadzu, Kyoto, Japan). For every calorimetric assay, ca. 10 mg of emulsion were weighed (using a microbalance) and directly submitted to slit heating (under nitrogen). A reference aluminium pan was also prepared by simply sealing an empty interior cavity. The samples were then heated from room temperature to 100 °C at a constant linear scanning rate of 5 °C/min, during which the amount of heat absorbed by the sample was recorded.

Thermodynamic stability of lipid nanovesicles depends upon their existing lipid microstructure, which is mainly determined by the structure and composition of the lipid core.

Thermogravimetric analysis was also performed (Shimadzu, Kyoto, Japan), since nanovesicles produced with Tween 80 and lecithin exhibited nearly the same melting behavior. The difference in the onset temperature of heat absorption was significantly displaced from 35.5 °C (in the case of nanovesicles produced with Tween 80) to 53.5 °C (in the case of nanovesicles produced with Tween 80 and lecithin).

Results and discussion

EVALUATION OF ANTIBACTERIAL ACTIVITY BY THE “SPOT” METHOD

In this research effort, development and optimization of lipid nanovesicles encasing bacteriophage-T4 was pursued to find a lipid with a minimal melting temperature, encompassing medium-to-long chain fatty acid molten fats was found most appropriate for the discoidal oily phase. A nanovesicle composition of 53.5 °C, the use of a low concentration of Tween 80, and low bacteriophage concentration were to be critical proportions for producing stable nanoveccles dispersions with diameters ranging from 115-145 nm and *Zeta Potential* ± 10. Inclusion of these multiple nanoveccles in lyophilic formulations for inhalational therapy of pharyngitis/laryngitis would possess inherent advantages, when compared with the current therapeutic approaches. Additionally, since in bacterial infections, in that bacteriophages are naturally host-specific with bacteriocidal activity, without any toxicological risk for humans.

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