Development of an inhalational therapeutic system to treat pharyngo-tonsillitis: a nanoencapsulation approach.

NOVA EN EVOLUÇÃO

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 agents of these tonsillar infections are Streptococcus pyogenes, a pathogen pathogen of the beta-hemolytic group A, which causes pharyngeal pharyngitis. The infection of these tonsils by such bacteria causes the tonsils to enlarge, which in turn can cause respiratory difficulties, since in this condition it is not easy to breathe through the mouth.

Experimental results and discussion

OPTIMIZATION OF THE NANOFORMULATION

Several variables were studied, viz.: lipid nature, poloxamer nature, soy lecithin concentration and tween 80 concentration.

Table 1. Optimization of processing conditions leading to an optimal nanof ormulation encasing T4 bacteriophage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>50 mg</td>
<td>65 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td>Poloxamer</td>
<td>50 mg</td>
<td>65 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td>Soy lecithin</td>
<td>50 mg</td>
<td>65 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td>Tween 80</td>
<td>50 mg</td>
<td>65 mg</td>
<td>75 mg</td>
</tr>
</tbody>
</table>

Replacement of the poloxamer by Lutrol F-127 led to a substantial decrease (from more negative towards less negative values) in the negativity of the Zeta Potential of the lipids nanovesicles.

In an additional experiment, using the optimal conditions (as determined in Table 1), the poloxamer F-68 was replaced by a lytic phage specific for Escherichia coli. This resulted in a significant decrease in the Zeta Potential, which is a measure of the stability of the nanovesicles. The increase in the Zeta Potential indicates a decrease in the stability of the nanovesicles, which is undesirable. Therefore, the optimal conditions determined in Table 1 are not suitable for the production of nanovesicles encasing a lytic phage specific for Escherichia coli.

Microbial ANALYSIS OF THE NANOFORMULATIONS

Calorimetric analysis was performed in a differentiating calorimeter (SETARAM, Kojyo, Japan). For each calorimetric cycle, ca. 10 mg of emulsion were used (weighed using a microsyringe) directly into the interior of high-pressure aluminum pans (10 mL, 5672010-124-0011), and duly sealed by pressure. A reference aluminum pan was also prepared by simply sealing an empty 10 mL aluminum pan.

The effect of changing the poloxamer was not very notorious in the amount of heat absorbed by the sample (see Figure 2). However, the thermal zone of heat absorption was significantly displaced from 35.5 °C (in the case of nanovesicles produced with Lutrol F-127) to 53.1 °C (in the case of nanovesicles produced with Lutrol F-68). The thermal stability of lipids micelles depends upon their existing lipid modification. Polymeric transitions after crystallization of triglycerol lipids are slower for longer chain triglycerides, as in our case, than for shorter chain triglycerides, whereas these transitions are faster for smaller sizes of crystals. The type of surfactant utilized in lipid nanovesicles formulation and their storage time affects the crystallinity of lipid nanovesicles and, consequently, its stability.

Conclusions

In this research effort, development and optimization of lipid nanovesicles encasing bacteriophage-T4 was pursued. A lipid with a mild melting temperature, encompassing medium-long chain fatty acids was found most appropriate for the differentiable oily phase. A homogenization temperature of 10 °C, the use of a low concentration of surfactant at the interface, and the use of 3% sodium deoxycholate as stabilizer were critical processing variables for producing stable nanovesicles dispersions with diameters ranging from 10-150 nm and Zeta Potential values between -40 and -30 mV. To evaluate the therapeutic potential of the nanovesicles, in vitro bioassay formulations for intestinal therapy of pharyngitis-like infections would possess inherent advantages, when compared with the current chemotherapeutic approach, if bacteriophage T4 were to be replaced by a lytic phage specific for Streptococcus pyogenes, in that bacteriophages are naturally harmless endotoxins with bacterial activity, without any toxicological risk for humans.