Bupivacaine-loaded nanoparticulated lipid carriers prepared with beeswax and melaleuca oil aiming the treatment of burn injuries: in vitro release study

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Abstract
Pain control in burn injuries is still inadequate and deserves improvement. For this, we have prepared an innovative formulation of nanoparticle lipid carriers (NLC) containing 1% bupivacaine (S75:R25, herein, BVC). The NLC were composed of beeswax and melaleuca oil. In vitro release tests showed a prolonged release (over 25 hours), confirming the preparation of a sustained drug-delivery system (DDS) for the local anesthetic. The formulation developed is promising for the next (in vivo tests, hydridization) steps of research, towards a long-lasting, topical painkiller.

Key words:
bupivacaine, nanoparticulated lipid carriers, melaleuca oil.

Introduction
The control of pain is a step of crucial importance in cases of burn injury. Pain is generated by the edema that causes compression of nerve endings on the epithelial surface, due to tissue injury1. The development of improved formulations for pain control in burn injuries is a requirement.

BVC is a local anesthetic agent widely used surgery, but it has a limited action, of 4-6 hours. Thus, to prolong BVC action time and to avoid several daily applications, it becomes interesting to encapsulate it in DDS such as nanostructured lipid carriers (NLC). NLC are composed of a lipid matrix characterized by a mixture of solid and liquid lipids2. The use of functional excipients may bring additional therapeutic advantages to the system. Beeswax has antioxidant properties, plus humectants and antimicrobial agents in its composition3. Melaleuca oil has antiseptic, antimicrobial, anti-inflammatory and antioxidant properties4.

The goal of this project is to study the release of BVC from NLC composed of beeswax, melaleuca oil and Pluronic F-68 (surfactant) loaded with 1% BVC, by using a Franz diffusion cell system.

Results and Discussion
NLC formulations were prepared by the emulsification-ultrasonication method2, with 10% beeswax, 4.5% melaleuca oil, 5% Pluronic F-68 and 1% enantiomeric excess S75:R25 bupivacaine (w/w). The formulations were characterized by Dynamic light scattering (DLS), Nanotanking analysis (NTA) and encapsulation efficiency (%EE), as shown in Table 1.

Table 1. Physical-chemical characterization of the prepared with bupivacaine (NLCBVC) or without (NLCFREE).

<table>
<thead>
<tr>
<th></th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Zeta (mV)</th>
<th>Particle s/mL</th>
<th>%EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLCFREE</td>
<td>233.7 ±1.14</td>
<td>0.127</td>
<td>-23.7</td>
<td>5.3 x 10^13</td>
<td>-</td>
</tr>
<tr>
<td>NLCBVC</td>
<td>238.7 ±2.2</td>
<td>0.142</td>
<td>-30.1</td>
<td>5.9 x 10^13</td>
<td>61.3 ±2.3</td>
</tr>
</tbody>
</table>

The in vitro release study was realized at 37±1 °C, in vertical Franz cell with Spectra/Por® dialysis membranes (12-14000 MW cutoff) against phosphate buffer pH 7.4. NLC formulations have a biodegradable matrix with temperature-dependent properties, that favors drug release2, as confirmed in this study. For the BVC solution 100% of the anesthetic was released after 4 hours, while the NLCBVC formulation showed a prolonged release profile, with total release after 24 hours (Fig. 1). The fraction of non-encapsulated drug (~40%) is responsible for the fast release (first 5 h) while the NLC respond for the sustained release rate observed till the end of the experiment.

Figure 1. In vitro release profile of BVC in solution (BVCFREE) and in the nanostructured lipid carriers (NLCBVC): 37 °C, n=4.

Conclusions
The in vitro release tests confirmed the encapsulation of BVC in the NLC prepared with beeswax and melaleuca oil, with sustained release longer than 24 h. This result, together with the good physical chemical properties (Table 1) endorses it for the next experiments aiming to produce a prolonged pain product to treat burn injuries.

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