Colloids and Surfaces B: Biointerfaces 164 (2018) 281–290

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Development of nanoparticles from natural lipids for topical delivery of thymol: Investigation of its anti-inflammatory properties

Thais P. Pivetta [a], Sandra Simões [b], Margarete M. Araújo [a], Tânia Carvalho [c], Caroline Arruda [d], Priscyla D. Marcato [a,*]

[a] Nanobiolab, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[b] Nanostructured Systems for Overcoming Biological Barriers group of iMed, ULisboa (Research Institute for Medicines), Faculdade de Farmacia, Universidade de Lisboa, Lisboa, Portugal
[c] Histology and Comparative Pathology Laboratory, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
[d] School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

ARTICLE INFO

Article history:
Received 19 June 2017
Received in revised form 4 January 2018
Accepted 26 January 2018
Available online 31 January 2018

Keywords:
Nanostructured lipid carriers
Thymol
Natural lipids
Topical delivery
Anti-inflammatory
Mice model

ABSTRACT

Wound healing involves the integration of biological and molecular events and, in case of chronic wounds, the use of drugs can be associated to side effects. Therefore, there is a search for alternatives therapeutics that encompass minimal toxicity. The use of natural compounds is an attractive approach for treating inflammatory disorders, wounds and burns. In this context, thymol has antimicrobial, antioxidant and antiseptic properties and is a promising compound in wound healing and inflammation management. However, essential oils and their constituents such as thymol present high volatility and can also easily decompose, thereby the encapsulation of these compounds into nanoparticles may be an efficient approach to modulate the release of the active ingredient, to increase the physical stability and to eventually reduce the toxicity. The aims of this work were to encapsulate thymol in nanostructured lipid carriers (NLCs) composed of natural lipids and assess its *in vivo* anti-inflammatory and antipsoriatic activity. The carrier containing thymol was produced by sonication method and showed 107.7 (±3.8) nm of size, zeta potential of −11.6 (±2.9) mV and entrapment efficiency of 89.1 (±4.2)%. Thymol-NLCs were incorporated into a gel and the final formulation presented rheological characteristics and pH suitable for topic application. In addition, the gel containing thymol-NLCs was tested *in vivo* on two different mouse models of skin inflammation, showing anti-inflammatory activity. Finally, this formulation was tested in an imiquimod-induced psoriasis mouse model and showed improved healing, compared to negative control. Therefore, thymol-NLCs is an interesting formulation for future treatment of inflammatory skin diseases.

© 2018 Published by Elsevier B.V.

1. Introduction

The skin is a protective barrier against external harmful agents as well as serve to maintain the temperature and other important functions [1,2]. This barrier is susceptible to several injuries and in many cases the injury occurrence can lead to skin inflammation [3]. There are various causes of inflammation including ultraviolet irradiation, microbial infection, immune reactions and physical damage and the most common signs are swelling, pain, erythema and increased heat. Some chronic diseases such as psoriasis can be developed due to inflammation and, in the case of chronic wounds, as those from psoriasis, it may involve the use anti-inflammatory agents such as steroidal and non-steroidal medications but some of these drugs are related with undesirable side effects. Therefore, there is a need to find new therapeutic alternatives that are less toxic [4,5].

The use of plants with medical purposes began a long time ago, including those used to treat inflammation and even nowadays it is an attractive approach to treat inflammatory diseases, wounds and burns [6]. Many extracts, oils and natural compounds have been studied regarding to possible biological activity in animal models and many of these studies presented anti-inflammatory and cicatrizing effects [7–9]. Riella et al. [10] studied the biological activities of thymol, a component of thyme oil from the plants of the *Thymus* genus, and concluded that this compound presents anti-inflammatory activity and can significantly improve the wound healing. Thus, some studies suggested that thymol is a compound with potential for the treatment of inflammatory pro-

* Corresponding author.
E-mail address: pmarcato@fcfrp.usp.br (P.D. Marcato).

https://doi.org/10.1016/j.colsurfb.2018.01.053
0927-7765/© 2018 Published by Elsevier B.V.

cesses and wound healing. Others studies showed that thymol presents antibacterial, antioxidant and anesthetic effects [11–13].

The constituents from essential oils present high volatility and can also decompose due to heat, humidity, oxygen or light. In addition to organoleptic and viscosity changes, oxidized terpenoids have also exhibited sensitization of the skin [14,15]. Therefore, the encapsulation of essential oils constituents represents a viable path for drug delivery, for the stability improvement and it is a manner to decrease volatility and toxicity of the drug. In this context nanotechnology appears as an innovative proposal for the encapsulation of bioactive compounds [16].

The use of lipid nanoparticles for drug delivery has gained the attention of many researchers due to the sustained release of the encapsulated drug and the safety of the raw materials [17]. Studies with solid lipid nanoparticles (SLNs) showed that these carriers have potential to be used on the treatment of the skin diseases by topical application [18]. Nanostructured lipid carriers (NLCs) succeeds the SLNs and were developed to enhance the encapsulation efficiency and to overcome the drug expulsion along time, that usually happened with SLNs [19]. NLCs presents others advantages such the increased permeation on skin and the occlusive effect that can improve the penetration of drugs besides the decrease the transepidermal water loss, increasing the skin hydration [20]. The encapsulation of anti-inflammatory drugs in NLCs was studied by Puglia et al. [21] and showed that the nanoparticles improved anti-inflammatory activity and allowed an extended delivery of the drugs on the epidermis.

Furthermore, lipid nanoparticles can be produced with natural lipids and this possibility presents the advantage of using lipids with biological properties themselves. Particularly, in this work we used Illipe butter which can promote skin hydration, and Calendula oil which has cicatrizing and anti-inflammatory effects [22–24]. These natural lipids were used to encapsulate thymol in NLCs and the thymol-NLCs systems was investigated for its anti-inflammatory activity.

2. Materials and methods

2.1. Materials

Thymol was purchased from Sigma-Aldrich (India) and Pluronic F68 was purchased from Sigma-Aldrich (Germany). Illipe butter was gently donated by Polytechno Indústrias Químicas Ltda. (Sweden). Calendula oil was purchased from Dermaclean (Brazil) and Carbopol® 940 from Acofarma (Spain). Croton oil and anthralin are from Sigma-Aldrich (USA). Aldara® was obtained from Meda (Sweden).

2.2. NLCs preparation

The method for NLCs preparation was hot emulsion followed by sonication. The oily phase containing Illipe butter and Calendula oil and the aqueous phase containing Pluronic F68 were heated separately to 50 °C, then the thymol was added to the oily phase. The aqueous phase was added to the oily phase and the system was sonicated during 10 min with a 13 mm probe (Sonics VCX 750, USA). The NLCs were formed after cooling the dispersion until 25 °C.

2.3. Characterization of the NLCs

2.3.1. Size and zeta potential

Size and polydispersity index (PdI) and the zeta potential were performed by dynamic light scattering (DLS) using Zetasizer Nano ZS90 (Malvern, UK). The measurements were made with samples diluted with 1 mM potassium chloride solution (1:100).

2.3.2. Encapsulation efficiency

To quantify thymol an ultraviolet-visible spectroscopy method was developed and validated (data not shown). Linearity, selectivity, precision, accuracy, detection, and quantification limits were evaluated as determined by Brazilian Sanitary Vigilance Agency [25] and ICH Guideline [26]. All samples were analyzed at the wavelength of maximum absorption of thymol in a spectrophotometer PG Instruments T70 + SW (UK).

Encapsulation efficiency (EE) was evaluated through an indirect method. Free thymol present in the dispersion was quantified after centrifugation of the NLCs dispersion with a microfilter (10,000 g/mol cutoff size, Millipore). The filtrate was diluted in ethyl alcohol (1:1) and then analyzed at the wavelength of 276 nm by the above referred method. The amount of thymol encapsulated in NLCs was calculated according to Eq. (1).

$$EE\,(\%) = \left(\frac{[Thymol]_{total} - [Thymol]_{not\quad encapsulated}}{[Thymol]_{total}} \right) \times 100 \qquad (1)$$

2.3.3. Morphology

The morphology of the empty NLCs was performed in a Shimadzu scanning microscope (SPM-9600 model). Nanoparticles dispersion was deposited on freshly cleaved mica plates and dried with air jet. The analysis was made using commercial silicon cantilevers (124 μm) and the images were obtained using a dynamic mode. The resonance frequency was 324–369 kHz.

2.4. Cell viability study

2.4.1. Cell line culture

A non-tumorigenic immortalized human keratinocytes cell line (HaCaT) from Rio de Janeiro Cell Bank (Brazil) was maintained in Dulbecco's Modified Eagle's Medium (DMEM), rich in glucose, supplemented with heat-inactivated 10% of fetal bovine serum (FBS) and 1% of antibiotics (10,000 units penicillin and 10 mg streptomycin per mL) in a CO_2 incubator (Panasonic, model MCO-170AICUVL-PA) at 37 °C and 5% of CO_2. The cells were harvested by treating with trypsin-EDTA 1X for 10 min, centrifuged at 1400 rpm, resuspended and plated in a 96-well culture plate in the amount of 5×10^4 cells per well and incubated for 24 h to adhere to the wells.

2.4.2. Treatment and neutral red uptake assay

The neutral red dye assay used to evaluate the cell cytotoxicity was adapted from Borenfreund and Puerner [27]. Dilutions of free thymol and thymol-NLCs were prepared in the range of 1.625–800 μM and empty NLCs was also tested. Samples diluted in medium without FBS (200 μL) were added to the wells and incubated for 24 h. Samples were removed from the plate, the wells were washed with phosphate-buffered saline (PBS) 1X and then 200 μL of neutral red dye (50 μg/mL in DMEM medium) was added and the plate was incubated for 3 h. After incubation, the medium containing the dye was removed and 200 μL of a fixing solution (1% of $CaCl_2$ dihydrate and 1% of formaldehyde in distilled water) was added. The solution was removed and the wells washed with saline solution 0.9% and then 200 μL of a solution containing 50% of absolute ethanol and 1% of acetic acid in distilled water was added to the wells and the plate was shaken gently during 10 min and the absorbance was analyzed in a microplate reader (Molecular Devices – SPECTRA max/Plus 384, UK) at 540 nm.

2.5. Gel samples preparation

For the animal studies, a gel formulation was developed for the topical administration of thymol (encapsulated in NLCs or in the free form) and for the empty NLCs. Carbopol® 940 was used as gelling agent (0.5% w/w) and glycerol (5% w/w) as humectant.

Download English Version:

https://daneshyari.com/en/article/6980615

Download Persian Version:

https://daneshyari.com/article/6980615

Daneshyari.com