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Research paper

Passive and active strategies for transdermal delivery using co-encapsulating nanostructured lipid carriers: *In vitro vs. in vivo* studies

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ABSTRACT

This work aimed at designing a formulation based on nanostructured lipid carriers (NLC) for transdermal co-administration of olanzapine and simvastatin, using passive and active strategies in a combined *in vitro*/*in vivo* development approach. NLC were prepared by two distinct methods, namely solvent emulsification–evaporation (SE/E) and high pressure homogenization (HPH). HPH was selected on the basis of a better performance in terms of drug loading and *in vitro* permeation rate. Several mathematical models were used to elucidate the release mechanisms from lipid nanoparticles. *In vitro* release kinetics was shown to be driven by diffusion, but other mechanisms were also present, and supported the feasibility of using NLC for sustained drug delivery. The *in vitro* skin studies showed that the chemical penetration enhancers, limonene and ethanol, added to the NLC formulations, promoted a synergistic permeation enhancement of both drugs, with olanzapine exhibiting a higher permeation than simvastatin. Transdermal administration to rats resulted in steady-state levels reached at around 10 h and maintained for 48 h, again with olanzapine exhibiting a better permeation rate. The pharmacokinetic parameters indicated that the NLC dispersion displayed a better *in vivo* performance than the gel, which was consistent with the *in vitro* results. These differences were, however, negligible in the flux values, supporting the use of gel as a final, more convenient, formulation. The *in vivo* experiments in rats correlated well with *in vitro* findings and revealed that the combined use of ethanol and limonene, incorporated in the NLC formulation, provided the main driving force for drug permeation. The Dermaroller® pretreatment did not significantly enhance drug permeation, supporting the use of passive methods as suitable for a transdermal delivery system. Furthermore, this work may provide a promising proof-of-concept for further clinical application in the treatment of schizophrenia and associated disorders, combined with dyslipidemia.

1. Introduction

The main challenge in transdermal drug delivery is to overcome the barrier of the stratum corneum, the outermost layer of the skin. Its unique composition of laterally overlapping, quasi-columnar stacks of corneocytes embedded in intercellular lipid multi-layers is crucial for the skin barrier function [1,2]. Numerous passive and active methods or the combination of both have been used to enhance the drug skin permeation [3]. Within passive methods, the combined use of lipid nanoparticles and chemical enhancers has emerged as a promising strategy to improve drug permeation [4]. The active microneedle-mediated delivery of nanoparticles into the skin has been regarded as an appealing method to potentiate drug transport through the skin [5,6].

In the present work, lipid nanoparticles or, more specifically, nanostructured lipid carriers (NLC) were used to simultaneously convey through the skin olanzapine (OL) and simvastatin (SV) (Fig. 1). Olanzapine is an atypical antipsychotic drug, extensively used in the treatment of schizophrenia and bipolar disorder [7], but long-term treatment is associated with metabolic adverse effects, in particular the alterations in the lipid profile [8,9]. Its association with simvastatin would prevent dyslipidemia and reduce

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cardiovascular risk [10,11]. Transdermal drug delivery systems (TDDS) were shown to improve compliance in patients suffering from chronic diseases [12]. Apart from the therapeutic relevance, the co-encapsulation in NLC, directed at transdermal delivery, is of fundamental interest in itself, with only a few studies reported in the literature [13–15]. In addition, both drugs gather physico-chemical properties that make them suitable as candidates for transdermal diffusion, such as a low molecular weight and melting point (OL, 312.4 and 195 °C; SV, 418.6 and 135–138 °C, respectively [16]), an adequate lipophilicity (OL log*P*, 2.8 [17]; SV log*P*, 4.7 [18]) and aqueous solubility (OL, 3-5 μg/mL [19]; SV, 30 μg/mL [16]).

NLC are colloidal systems, made of a blend of liquid and solid lipids, stabilized in an aqueous surfactant(s) solution [20]. Their chemical similarity to skin lipids, stemming from the common hydrophobic character, the existence of a solid matrix, the high specific surface area linked to their nanometer-scale size, and the biocompatibility make these carriers suitable for long-term skin administration. In the present study, solvent emulsification–evaporation and high pressure homogenization were investigated for the preparation of the NLC. The respective performance for transdermal OL and SV delivery was investigated *in vitro* and *in vivo*, in association with permeation enhancers and the skin pre-treatment microneedle roller device (Dermaroller®).

2. Materials and methods

2.1. Chemicals

Simvastatin was kindly provided by Labesfal (Santiago de Besteiros, Portugal). Olanzapine was purchased from Zhejiang Myjoy (Hangzhou, China). Simvastatin hydroxy acid ammonium salt and olanzapine-D3 were purchased from @rtmolecule (Poitiers, France). Glyceryl tripalmitate (tripalmitin, T8127), phosphate buffer saline (PBS) pH 7.4, polyvinyl alcohol 87–89% hydrolyzed (PVA, typical MW 13.000–23.000), polysorbate 80 (Tween® 80) and polyethylene glycol 400 (PEG 400) were purchased from Sigma. Oleic acid and limonene were acquired from Fluka. Carbopol® Ultrez 10 NF was kindly provided by Lubrizol (Quimidroga, Barcelona, Spain). The microneedle device Dermaroller® MC905 (equipped with 192 stainless steel microneedles of 500 μm) was bought from Distrimed S.a.r.l. (Luxembourg). All chemicals used were of analytical grade, and solvents were of high-performance liquid chromatography (HPLC) grade.

2.2. Preparation of NLC by solvent emulsification-evaporation method

The production of NLC by solvent emulsification–evaporation (SE/E) was based on a previously optimized method for the

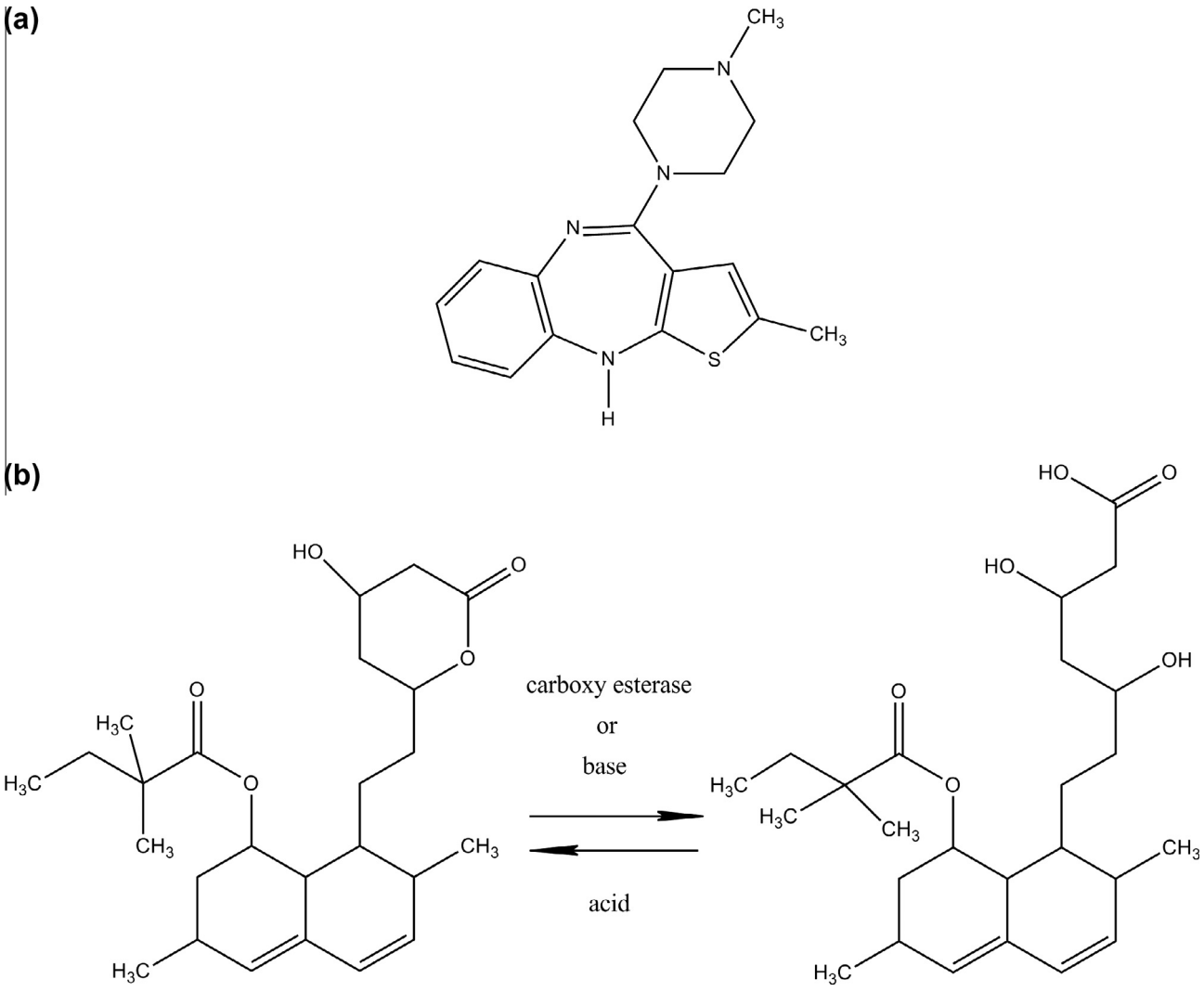

Fig. 1. Chemical structures of (a) olanzapine (OL) and (b) simvastatin (SV) with the respective active hydroxy acid metabolite, simvastatin acid (SVA).