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Development of a ropivacaine-loaded nanostructured lipid carrier formulation for transdermal delivery

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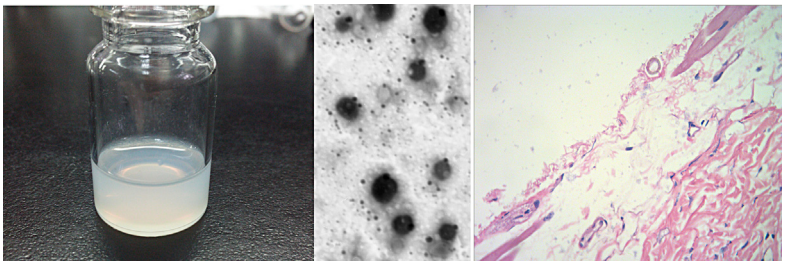
HIGHLIGHTS

- Novel ropivacaine loaded nanostructured lipid carriers (RPV-NLCs) were prepared.
- RPV-NLCs showed improved permeation flux with J_s of 5.386 μg cm^{-2} h^{-1}.
- RPV-NLCs achieved a C_{max} value of 5.98 μg/mL in blood.
- The permeation mechanism was studied by skin histopathology and DSC.

GRAPHICAL ABSTRACT



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ABSTRACT

The main objective of this study was to evaluate the potential of ropivacaine-loaded nanostructured lipid carriers (RPV-NLCs) as a transdermal delivery system. RPV-NLCs were prepared by the method of emulsion evaporation–solidification at low temperature. The average entrapment efficiency and drug loading of the optimized RPV-NLCs were 81.45 ± 2.16% and 2.95 ± 0.37%, respectively. The average particle size was 203.5 ± 1.2 nm and the zeta potential was −40.2 ± 3.3 mV. The results of *in vitro* permeation study showed that RPV-NLCs could promote the permeation of RPV through skin to achieve transdermal delivery with Q_n of 345.6 ± 12.4 μg cm^{-2}. In the mice writhing test, RPV-NLCs provided analgesic effect by reducing the writhing response with an inhibition rate of 89.1% compared to the control group. In addition, the mechanism of permeation enhancement for NLCs investigated by histopathology study and DSC analysis showed NLCs could interact with stratum corneum, weaken the barrier function and facilitate drug permeation. In conclusion, NLCs could be a promising vehicle for transdermal delivery of RPV.

1. Introduction

Ropivacaine is a new, long-acting aminoamide local anesthetic, which is the first enantiomerically pure local anesthetic and exists as the *S*-enantiomer [1]. In healthy volunteers, ropivacaine was proved less prone to produce central nervous system and cardiovascular changes than its homolog bupivacaine after intravenous infusion (ropivacaine caused less central nervous system symptoms and was at least 25% less toxic than bupivacaine in regard to the dose tolerated) [2]. Furthermore, researches done by Rosenberg [3] showed that ropivacaine at low concentration

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produced a profound block of both Aδ and C fibers. More meaningful, Wildsmith [4] demonstrated that ropivacaine blocked C fiber faster than A fiber. The property of the great degree of differential block between nerve fibers responsible for transmission of pain (Aδ fibers) and those that control motor function (Aβ fibers) was considered to offer considerable clinical advantages in proving analgesia with minimal motor block [5]. Actually, ropivacaine showed great separation of motor and sensory block in clinical trials. The clear advantages of low toxicity and sensory-motor differential block makes ropivacaine well suited for postoperative analgesia [2]. Due to the aforementioned virtues, ropivacaine has been introduced into clinic as an alternative since 1996 in the form of Naropin, which should be administed via injection. However, the intravenous infusion is offering poor patient convenience since it is found to be painful and risky [6]. Not only that, the side effects induced by intravenous infusion including the risk of systemic toxicity related to overdosing or too fast injection speed is high. Thus, in order to provide a feasible and safer alternative to Naropin®, developing novel formulation forms which are both capable for transporting ropivacaine in a suitable administration route and achieving controlled-release makes great sense.

Skin as the largest organ of the human body with considerable surface area has been historically used for the topical delivery of compounds [7]. It may provide a feasible administration route for delivering ropivacaine. Transdermal drug delivery system can control the release of the drug into the systemic circulation, avoiding the major fluctuations of plasma levels and enable a steady blood level profile, reducing systemic side effects. In addition, transdermal drug delivery is easy for application and for practical management with user friendly, improving patient convenience for postoperative analgesia. Lipid-based colloid carriers have considerable potential in topical delivery of drugs due to their distinctive properties [8]. For example, the first marketed transdermal therapeutic for the whole body treatment was introduced in 1980, containing scopolamine [9]. A few other, liposome-based dermal products followed. The Hepaplus liposome gel (hexal pharma) is a current product at least in some countries [9]. All the products proved the feasibility of delivering drug into systemic circulation via proper lipid particles carriers.

Nanostructured lipid carriers (NLCs), as one of the lipid particles carrier, was introduced by Müller et al. [10] at the end of the 1990s and had caught special attention and truly deserved a seat. NLCs belong to the second generation of lipid nanoparticles [11]. They are mixtures of solid and fluid lipids. The fluid lipid phase was reported to be embedded into the solid lipid matrix [12] or to be localized at the surface of solid platelets and the surfactant layer. The combined application of spatially different lipids leads to general imperfections in the crystal and favors a less-ordered crystalline structure or an amorphous solid structure, which provides more room for accommodation of encapsulated drugs. Hence, compared to its predecessor SLNs, NLCs preparations can overcome some fatal drawbacks like expulsion which is caused by the ongoing crystallization or transformation of the solid lipid during the stored procedures [13]. Specially, the colloid character makes NLCs advantageous as transdermal drug delivery. Firstly, the small size of the NLCs ensures close contact to the stratum corneum of skin. They are able to attach themselves to the skin surface, promoting adhesiveness and increasing hydration. In addition, as the ingredient of NLCs, lecithin plays a role in transdermal drug delivery. They interact with the outermost layers of stratum corneum, allowing lipid exchange or fluidization within intercellular lipid domain. All these effects facilitate drug permeation into the deep skin.

Thus, aims to develop novel external preparation to alter traditional intravenous infusion, the present study designed ropivacaine-loaded nanostructured lipid carriers (RPV-NLCs) and characterized its potential for transdermal delivery. *In vitro* drug permeation through excised mice skin was carried out and the pharmacodynamics study was evaluated by writhing test. Furthermore, the permeation mechanism was evaluated by skin histopathology and differential scanning calorimetry (DSC) analysis.

2. Materials and methods

2.1. Materials

Ropivacaine (RPV) was purchased from Jinan Dexinjia Pharmaceutical Co. Ltd. (Jinan, China). Soya lecithin (SL) was provided by Shanghai Taiwei Pharmaceutical Co. Ltd. (Shanghai, China). Glyceryl monostearate (GMS) was obtained from Shanghai Chemical Reagents Co. Ltd. (Shanghai, China) and stearic acid (SA) by Beijing Chemical Reagents Co. Ltd. (Beijing, China). Media chain triglyceride (MCT) was purchased from Tieling Beiya Medicinal Oil Co. (Tieling, China). All other chemicals were of analytical purity and commercially available.

2.2. Preparation of RPV-NLCs

Many different methods have been described in the literature for production of NLCs. In this research, the RPV-NLCs were prepared by the method of emulsion evaporation–solidification at low temperature according to the previous report [14]. Briefly, RPV (30 mg) and lipids (200 mg) were completely dissolved in 3 mL of chloroform/acetone (1:1 v/v), and then the resultant organic phases was dispersed dropwise into 15 mL of 4% Solutol HS15 (w/v) aqueous solution, under mechanical agitate with 600 rpm. Subsequently, the aforementioned mixture was emulsified for 4 h at 70 °C to insure the organic phase was effectively removed. After that the obtained semi-transparent nanoemulsion was transferred into 25 mL of cold distilled water (0–2 °C), stirring for 2 h at 0 °C. Eventually, the dispersion was centrifuged at 4000 rpm for 10 min so as to remove aggregates of non-encapsulated RPV. Finally, the homogeneous NLCs dispersion was obtained from the supernatant.

2.3. Drug encapsulation efficiency (EE) and drug loading (DL)

The drug content in the supernatant after centrifugation was measured by HPLC. Briefly, analysis was performed by using an Agilent 1200 HPLC system (Agilent, America). Chromatographic separation was achieved on a BDS C18 column (250 × 4.6 mm^2, 5 μm, Yilite Co., Ltd., Dalian, China). The mobile phase was composed of acetonitrile, 0.053 mol/L phosphate buffer and triethylamine in a volume ratio of 60: 40: 0.05. Prior to use, the mobile phase was filtered through a 0.45 μm hydrophilic membrane filter. Detection was performed at a wavelength of 225 nm. The drug encapsulation efficiency (EE) and drug loading (DL) percentage of RPV-LNC were then calculated from the following formulas:

$$EE\% = \frac{W_{loaded}}{W_{added}} 100$$

$$DL\% = \frac{W_{loaded}}{W_{total}} 100$$

where, "W_{loaded}" is the amount of RPV encapsulated in NLCs and the "W_{added}" is the amount of RPV detected in the RPV-NLCs dispersion. The "W_{total}" stands for the weight of NLCs.

2.4. Optimization of preparation parameters

During the preparation procedure, it was proved that the amount of RPV, HS 15, SL, MCT and the ratio of GMS to SA have great effects on the properties of RPV-NLCs. In order to improve