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Original article

Local anaesthetic pain relief therapy: *In vitro* and *in vivo* evaluation of a nanotechnological formulation co-loaded with ropivacaine and dexamethasone

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ABSTRACT

Combination therapy is frequently applied to anesthesia and analgesia for its benefits, which includes prolonged analgesia following peripheral nerve blockade, and reduced side effects. The aim of this study was to develop chitosan (CH) coated poly(ε-caprolactone) (PCL) nanoparticles to co-deliver ropivacaine (RPV) and dexamethasone (DEM) (RPV/DEM CH-PCL NPs) for the prolongation of anesthesia and pain relief. In the present study, RPV/DEM CH-PCL NPs were fabricated. The properties of CH-PCL NPs were evaluated for their particle sizes, zeta potential, drug loading capacity and *in vitro* drug release profile. *In vitro* skin permeation and *in vivo* therapeutic effect in an animal model were further investigated. The results showed that the NPs was around 190 nm, with PDI of less than 0.20. The zeta potentials of NPs were about 36 mV. *In vitro* drug release of both RPV and DEM from NPs complied with sustained behaviors. All of the drugs loaded NPs samples studied exhibited no obvious L929 cells cytotoxicity. *In vitro* skin penetration profiles showed the amount of RPV permeated through the skin from NPs was significantly higher than free RPV. RPV and DEM co-loaded NPs induced remarkably better anesthetic effect than non DEM loaded RPV CH-PCL NPs. The results suggested that adding a small dosage of DEM could improve the anesthesia efficacy of RVP to a large content. The resulting formulation could be applied as a promising anesthesia system for local anesthetics therapy.

1. Introduction

There are over 200 million people undergoing major surgery annually over the world [1]. After surgery, efficient postoperative pain management plays an important role in improving life quality as well as reducing the risk of developing chronic pain [2]. However, pain relief after major surgery is difficult to achieve and makes the postoperative care complicated. Recently, more and more attention in both scientific and clinical researches has been paid to the use of local anesthetics in the field of postoperative analgesia [3,4].While, the duration of action of plain local anesthetic (LA) agents is limited. There are several effective strategies to prolong LA action, including using adjuvants to LA agents such as opioids and dexamethasone, and encapsulation of LA agents with nanocarriers [5–7].

Ropivacaine (RPV) is a member of the amino amide class of local anesthetics and supplied as the pure S-(−)-enantiomer [8]. It has been approved for use in surgical anesthesia and acute pain management, local infiltration and peripheral nerve blocks. Compared with lidocaine, RPV has been shown to cause longer peripheral neural blockade [9,10]. Compared with bupivacaine, RPV has similar efficacy, lower CNS and cardiotoxicity and lower propensity for motor block [7].

Combination therapy is frequently applied to anesthesia and analgesia for its benefits, which include prolonged analgesia following peripheral nerve blockade, and reduced side effects [11]. Numerous randomized controlled trials and meta-analyses have reported the pros and cons of combination therapy of local anesthetics and adjuvants [12]. Results have demonstrated that perineural buprenorphine, clonidine, dexamethasone, dexmedetomidine, and magnesium most consistently prolonged peripheral nerve blocks [12,13]. Among them, dexamethasone (DEM) is widely utilized to improve the quality and duration of peripheral never blocks over LA alone [5].This is thought to be mediated by the reduction of ectopic neuronal discharge, the attenuation of inflammatory mediator release, and the inhibition of potassium channel-mediated discharge of nociceptive C-fibres [14–16]. Moreover, more and more researches have indicated that RPV plus DEM is a regimen for the anesthesia of transabdominal hysterectomy, major

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abdominal surgery, and arthroscopic shoulder surgery [17–20].

Topical anesthetics are widely employed in clinical procedures in order to improve patient compliance like reducing the discomfort caused by needle insertion and making self-administration easier [21]. Nanoparticles offer a number of advantages for topical anesthetics, including controlled drug release, dual or multi-agents encapsulation, and increased drug penetration and permeation through the skin [22,23]. Some evidence showed that polymeric nanoparticles such as poly(ε-caprolactone) (PCL), poly lactic-co glycolic acid (PLGA), chitosan, etc can successfully deliver drugs to penetrate the skin [24]. Chitosan (CH) is a cationic polymer and widely used as drug delivery because of its non-toxicity, biodegradability and biocompatibility [25]. The positive-charged property of CH makes retention and permeation through the negatively-charged membrane efficiently [26,27]. Foley et al., used CH base nanoparticles to deliver RPV and DEM [7]. They proved that a nano-system composed of chitosan, ropivacaine and dexamethasone would result in controlled anesthetic drug delivery and sustained anesthetic effects *in vivo*.

The main goal of this study was to develop chitosan coated PCL nanoparticles to co-deliver RPV and DEM (RPV/DEM CH-PCL NPs) for the prolongation of anesthesia and pain relief. The properties of CH-PCL NPs were evaluated for their particle sizes, zeta potential, drug loading capacity and *in vitro* drug release profile. *In vitro* skin permeation and *in vivo* therapeutic effect in animal model were further investigated.

2. Material and methods

2.1. Chemicals and reagents

CH (average Mw 218 KDa), PCL (average Mw 14 KDa), RPV, DEM, dichloromethane (DCM), Roswell Park Memorial Institute-1640 (RPMI-1640), and fetal bovine serum (FBS) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Cell counting kit-8 (CCK-8) was purchased from MedChem Express (Monmouth Junction, NJ, U.S.A.). All other chemicals and reagents were of analytical grade or high performance liquid chromatography (HPLC) grade and used without further purification.

2.2. Cells and animals

Mouse fibroblast L929 cells (L929 cells) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Healthy Sprague-Dawley rats (weighing 250 to 300 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animal experiments were approved by the Medical Ethics Committee of Jining Medical College (No. JNMC20170506-001).

2.3. Preparation of RPV/DEM CH-PCL NPs

RPV and DEM co-loaded PCL NPs (RPV/DEM PCL NPs) were prepared by using modified double emulsion technique [28]. Briefly, RPV and DEM were dissolved in 0.5 mL of purified water by vortex mixing. PCL was dissolved in 2 mL of DCM. Then the drugs solution was emulsified in PCL contained DCM using a probe sonicator for 60 s under ice bath. The formed primary emulsion was immediately mixed with an aqueous solution of 1% PVA followed by probe-sonication for 3 min. The DCM was then removed by stirring the mixture at 500 rpm for 4 h. The RPV/DEM PCL NPs were separated by using a centrifuge at 20,000 rpm for 15 min at 4 °C.

RPV/DEM CH-PCL NPs (Fig. 1) were obtained by incubating a certain volume of suspensions of RPV/DEM PCL NPs with an equivalent volume of 2 mg/mL chitosan in 0.5% acetic solution for 2 h at room temperature. The resulted RPV/DEM CH-PCL NPs were centrifuged at 20,000 rpm for 15 min at 4 °C. RPV single drug loaded CH-PCL NPs (RPV CH-PCL NPs) were prepared by the same method without adding DEM. Blank CH-PCL NPs without RPV or DEM were prepared by the same method without adding RPV and DEM.

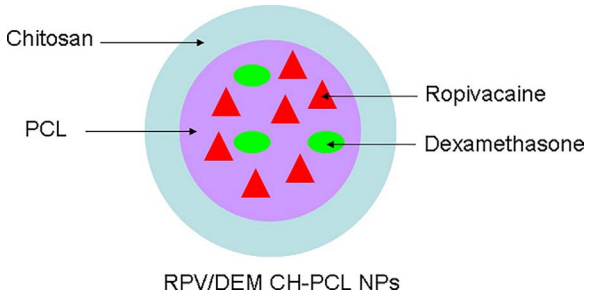

Fig. 1. Schematic diagram of RPV/DEM CH-PCL NPs.
Abbreviations: RPV, ropivacaine; DEX, dexamethasone; CH, chitosan; PCL, poly(ε-caprolactone); NPs, nanoparticles.
Notes: Data is presented as means ± standard deviation, n = 3.

All the obtained NPs dispersions were stabilized using a Freeze Dryer (Laboratory −80 °C vertical low-temperature freeze-drying machine, TF-FD-1L, Shanghai Tianfeng Industrial Co. Ltd, Shanghai, China). Detailed lyophilization procedure: Pre-freezing, add mannitol (1:1, w/w with NPs) into the NPs suspension, set the temperature to −80 °C and maintain 4 h. Evacuation, when the temperature reached −80 °C, evacuate the lyophilizer until below 0.4 mbar. Sublimation drying: raise the temperature to −20 °C for 6 h, −15 °C for 10 h and −10 °C for 6 h. Desorption drying: raise the temperature to 20 °C for 8 h, set the vacuum degree 0.15 mbar and maintain 6 h. Then raise the vacuum degree to 0.01 mbar and maintain for 8 h. Increase the pressure slowly and insulation drying for several hours. The size, PDI and EE of the nanoparticles remained stable for 2 month post lyophilization. The compositions of different formulations are presented in Table 1.

2.4. Characterization of RPV/DEM CH-PCL NPs

The various kinds of NPs samples were diluted 100 times in ultra-purified water and analyzed by Dynamic Light Scattering, using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, U.K.) for the characterization of average vesicle size, polydispersity index (PDI) and zeta potential [29].

Drug loading content (DL) and entrapment efficiency (EE) of NPs were determined [30]. The NPs dispersions were centrifuged in a centrifugal filter device to remove the nonentrapped drugs. RPV content was analyzed by Reverse-Phase High-Performance Liquid Chromatography (HPLC) [7].Specifically, 10 μL of sample was injected into a mobile phase of 50% phosphate buffer (PBS) and 50% acetonitrile (0.1% TFA) at a flow rate of 1 mL/min and passed over a 5 μm reverse-phase column. Ropivacaine elution was detected by an Agilent 1100 diode array detector at 262 nm. DEM content was analyzed f by UV absorbance at 242 nm.

2.5. In vitro drug release from NPs

In vitro release of RPV and DEM from NPs was investigated using a dialysis method (MWCO, 100 kDa) at 37 °C [31]. The drug release was

Table 1
The compositions of different formulations.

Ingredients	RPV (mg)	DEM (mg)	CH (mg)	PCL (mg)
Blank CH-PCL NPs	/	/	100	200
RPV CH-PCL NPs	100	/	100	200
RPV/DEM CH-PCL NPs-1	100	0.5	100	200
RPV/DEM CH-PCL NPs-2	80	1	100	200
RPV/DEM CH-PCL NPs-3	60	1.5	100	200
RPV/DEM CH-PCL NPs-4	40	2	100	200
RPV/DEM CH-PCL NPs-5	20	2.5	100	200