Biomaterials 34 (2013) 2539–2546

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

A chitosan thermogel for delivery of ropivacaine in regional musculoskeletal anesthesia

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ARTICLE INFO

Article history:
Received 21 November 2012
Accepted 29 December 2012
Available online 12 January 2013

Keywords:
Chitosan
Thermogels
Anesthetics
Nanoparticles
Pain management

ABSTRACT

Postoperative pain within the first days following musculoskeletal surgeries is a significant problem for which appropriate management correlates to positive clinical outcomes. While a variety of pain management modalities are currently used for postoperative pain, an optimal strategy has yet to be identified. Utilizing local anesthetics to convey analgesia through neural blockade represents a promising approach to alleviate postoperative pain. Unfortunately, local anesthetics are often associated with short half-lives, local tissue site reactions, and systemic toxicity. Drug delivery systems such as liposomes, microparticles, and nanoparticles have been previously utilized to extend analgesia, but these systems can easily diffuse from the injection site. In order to overcome this limitation a combination of drug delivery technologies were utilized. Ropivacaine base nanoparticles were fabricated and entrapped with dexamethasone using a chitosan thermogel delivery system in order to enhance neural blockade. Using a rat sciatic neural blockade model, this system was able to limit sensory function and motor function for up to 48 h. This approach utilized a low solubility drug, a drug action enhancer, nanoparticles, and a thermogel matrix together to yield a multi-faceted delivery system capable of providing moderate-term pain management.

1. Introduction

A variety of musculoskeletal procedures including fracture fixation, total knee replacement, total hip replacement, and rotator cuff repair result in considerable postoperative pain for up to 48 h following surgery [1]. A variety of medications are available to patients dealing with postoperative pain such as acetaminophen, non-steroidal anti-inflammatory drugs, corticosteroids, anesthetics, and opioids. Recently, the use of local anesthetics to provide postoperative analgesia has received considerable scientific and clinical interest [2,3]. While promising, local anesthetics possess short durations of action so neural blockade enhancers like dexamethasone are often utilized in combination with these drugs [4,5]. A variety of local anesthetics are available on the market with the amino-amide drugs lidocaine, bupivacaine, and ropivacaine being three of the most commonly used. Bupivacaine and ropivacaine have been shown to cause longer peripheral neural blockade (4.5–12 h) than lidocaine (1–2 h) [6–8]. In order to extend the activity of these fast acting local anesthetic drugs, a neural blockade enhancer like dexamethasone is often included. Bupivacaine is a widely used long-acting anesthetic, but is associated with significant cardiotoxicity and neurotoxicity [9,10]. Ropivacaine, the propyl analog of bupivacaine, has been shown to be less cardiotoxic and neurotoxic than bupivacaine [11,12] while maintaining a similar duration of action [13]. Additionally, ropivacaine possesses lower lipid solubility [14] and vasodilation [15] than bupivacaine allowing for better retention in the local environment it is delivered. While promising, local anesthetics require continuous infusion to maintain desirable postoperative pain management [16] which can lead to significant issues such as adverse local tissue reactions [17] and systemic toxicity [18,19].

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http://dx.doi.org/10.1016/j.biomaterials.2012.12.035

The use of biomaterials to achieve controlled release of local anesthetics has the potential to yield a safe, localized, long-acting postoperative pain management system. A number of biomaterial-based carriers have been previously explored including liposomes [5], microparticles [20], and nanoparticles [21]. These materials are capable of extending the release of encapsulated drugs, but can freely diffuse from the injection site. Thermogels are a class of biomaterials capable of existing as injectable solutions at room temperature that transition to colloidal gels *in situ* as they warm to body temperature. These materials are ideal for sustained, localized anesthetic delivery since the drug can be easily dispersed and then injected into the patient where upon gelation, the thermogel will maintain drug delivery at the injection site. Thermogels can be composed of a variety of different synthetic [22,23] or natural polymers [24,25]. Chitosan is a linear polysaccharide that is synthesized by deacetylating chitin, a structural element found in the exoskeleton of crustaceans. It can be crosslinked by inorganic phosphate salts to become a thermogelling solution which has shown promise as an injectable drug delivery vehicle for over ten years [26,27]. Chitosan thermogels are non-cytotoxic [28], can be tailored to gelate quickly [29] while capable of maintaining sustained payload delivery [28,30], and degrade slowly over time into bioresorbable products [31], making them promising local anesthetic delivery vehicles.

In the present study, chitosan thermogels were utilized to deliver ropivacaine base nanoparticles with and without dexamethasone. Our hypothesis was that a gel system composed of chitosan, ropivacaine and dexamethasone would result in controlled anesthetic drug delivery and sustained anesthetic effects *in vivo*.

2. Materials and methods

2.1. Materials

Ropivacaine hydrochloride was a generous gift from AstraZeneca (London, United Kingdom). Dexamethasone microparticles (1.69 ± 0.89 μm) and ammonium hydrogen phosphate (AHP) were purchased from Sigma–Aldrich (Saint Louis, MO). Ultrapure biomedical grade chitosan (>74.5% deacetylation) was obtained from Biosyntech (Quebec City, Canada). Deionized, distilled water (ddH_2O) was generated by a Millipore Milli-Q integral water purification system (Billerica, MA).

2.2. Aqueous precipitation of ropivacaine base

Crystalline ropivacaine hydrochloride (3 g) was added to 75 mL ddH_2O and mixed until completely dissolved. Ammonium hydroxide (6.52 mL of 14 N, 10× molar equivalent) was added to the ropivacaine hydrochloride solution to induce alkaline precipitation of ropivacaine base (Fig. 1). The precipitated base was filtrated and washed extensively with ddH_2O to remove all associated ammonium hydroxide. The resulting powder was lyophilized and stored under desiccant until further use. Ropivacaine hydrochloride and ropivacaine base morphology was evaluated by scanning electron microscopy (SEM, JEOL 6320F Field Emission Scanning Electron Microscope, Tokyo, Japan) and analyzed by the image analysis software ImageJ (NIH, Bethesda, Maryland).

2.3. Preparation of thermogelling chitosan solution

A 1.6% w/v chitosan stock solution was made by mixing chitosan into a 0.25% acetic acid solution with magnetic stirring in an ice bath until translucent. The resulting chitosan solution was steam sterilized at 121 °C for 20 min. Following sterilization, the solution was kept at 4 °C until further use. A 60% AHP solution was prepared, subjected to filter sterilization, and chilled to 4 °C. The thermogelling chitosan formulation was prepared by adding 9 μL of the AHP solution dropwise to 0.5 mL of the chitosan solution in an ice bath and mixing for 2–5 min until the solution became translucent. The solution was kept on ice to prevent premature gelation.

2.4. In vitro ropivacaine release from chitosan thermogel

Ropivacaine base (75 mg) was added to 0.5 mL thermogelling chitosan formulation and mixed by magnetic stirring in an ice bath. Once the ropivacaine was thoroughly mixed, the solution was rapidly injected into a dialysis bag and sealed. The dialysis bag was incubated in a 1.5 L phosphate-buffered saline (PBS) bath at 37 °C. With ropivacaine base solubility in water (0.253 mg/mL), the bath volume was greater than 5 times the saturation volume ensuring sink conditions. Samples (1 mL) were extracted from the PBS bath at time points of interest over a 7 day period and replaced with equal volumes of PBS.

Samples were analyzed for ropivacaine content by Reverse-Phase High-Performance Liquid Chromatography (HPLC, Agilent 1100 Series, Agilent Technologies, Wilmington, DE). Specifically, 10 μL of sample was injected into a mobile phase of 50% PBS and 50% acetonitrile (0.1% TFA) at a flow rate of 1 mL/min and passed over a 5 um reverse-phase column (Zorbax Eclipse SDB-C18, Agilent Technologies, Wilmington, DE). Ropivacaine elution was detected by an Agilent 1100 diode array detector at 262 nm. Samples taken from a PBS bath exposed to an equivalent amount of unloaded chitosan gel were used to remove any contaminating effects from the chitosan itself. A standard curve of ropivacaine in PBS was generated over the range of 0.2–20 μg/mL and used to convert absorbance to concentration. A cumulative release profile was generated by normalizing the data against the total amount of encapsulated ropivacaine and reported as fractional drug release.

2.5. In vitro dexamethasone release from chitosan thermogel

Dexamethasone (1 mg) was added to 0.5 mL thermogelling chitosan formulation and mixed by magnetic stirring in an ice bath. Once the dexamethasone was thoroughly mixed, the solution was rapidly injected into a dialysis bag and sealed. The dialysis bag was incubated in a 1.5 L phosphate-buffered saline (PBS) bath at 37 °C. With dexamethasone solubility in water (0.1 mg/mL), the bath volume was greater than 150 times the saturation volume ensuring sink conditions. Samples (1 mL) were extracted from the PBS bath at time points of interest over a 7 day period and replaced with equal volumes of PBS.

Samples were analyzed for dexamethasone content by UV absorbance (UVmini-1240, Shimadzu, Kyoto, Japan) at 242 nm. Samples taken from a PBS bath exposed to an equivalent amount of unloaded chitosan gel at corresponding time points were used to remove any contaminating effects from the chitosan itself. Absorbance was converted to concentration with the use of a standard curve (0.2–20 μg/mL). A cumulative release profile was generated by normalizing the data against the total amount of encapsulated dexamethasone and reported as fractional drug release.

2.6. Animals

Sprague–Dawley, specific pathogen free, female rats (250–350 g in weight) were purchased from Charles River Laboratories (Wilmington, MA) and housed in ventilated cage systems. Animals were maintained in a temperature and humidity-controlled vivarium, on a 12/12 h light/dark cycle, with access to rodent chow and water *ad libitum*. All animal study protocols were approved by the Institutional Animal Care and Use Committee at the University of Virginia.

2.7. Sciatic nerve blockade

Baseline assessments were performed on all animals for at least 2 days prior to surgery to confirm normal sensory function, motor function, and body weight. The animals were anesthetized using isoflurane anesthesia, and under aseptic conditions, a small incision was made on the lateral aspect of the upper right thigh. The common sciatic nerve was exposed by blunt dissection of the biceps femoralis

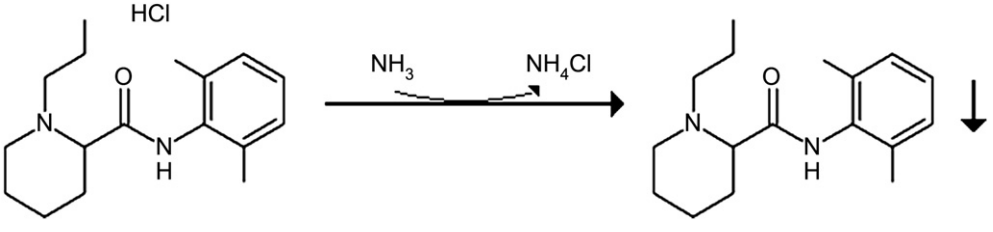

Fig. 1. The addition of ammonia to ropivacaine hydrochloride (left) in solution causes sequestering of the hydrochloride yielding insoluble ropivacaine base (right) that rapidly precipitates out of solution.