Potential Avoidance of Adverse Analgesic Effects Using a Biologically "Smart" Hydrogel Capable of Controlled Bupivacaine Release

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ABSTRACT: Acute pain remains a tremendous clinical and economic burden, as its prevalence and common narcotic-based treatments are associated with poorer outcomes and higher costs. Multimodal analgesia portends great therapeutic promise, but rarely allows opioid sparing, and new alternatives are necessary. Microparticles (MPs) composed of biodegradable polymers [e.g., poly(lactic-co-glycolic acid) or PLGA] have been applied for controlled drug release and acute pain treatment research. However, foreign particles' presence within inflamed tissue may affect the drug release or targeting, and/or cause a secondary inflammatory reaction. We examined how small alterations in the particulate nature of MPs affect both their uptake into and subsequent activation of macrophages. MPs composed of PLGA and chitosan (PLGA–Chi) loaded with bupivacaine (BP) were engineered at different sizes and their opsonization by J774 macrophages was assessed. Uptake of PLGA–Chi by macrophages was found to be size dependent, but they were not cytotoxic or proinflammatory in effect. Moreover, encapsulation of MPs in a thermoresponsive loading gel (pluronic F-127) effectively prevented opsonization. Finally, MPs displayed sustained, tunable release of BP up to 7 days. These results demonstrate our ability to develop a drug delivery system capable of controlled release of local anesthetics to treat acute/subacute pain while concurrently avoiding enhanced inflammation. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3724–3732, 2014

Keywords: pain; drug delivery; controlled release; inflammation; biomaterials; microparticles; injectables

INTRODUCTION

Acute postoperative pain is a complex, still unresolved phenomenon strictly related to postoperative morbidity and to a chronic postsurgical pain syndrome.[1,2] The impact of uncontrolled acute or subacute pain is far-reaching as well, with numerous psychosocial and functional consequences that significantly impact overall patient quality of life.[3–7] Regional analgesia and multimodal therapy, significantly diminishing opioid requirements, and adverse effects incidence have transformed postoperative care, improving patient's outcome and enhancing their recovery.[8–13] Continuous peripheral local anesthetic administration is a rational approach to control pain as part of such multimodal strategies to reduce both peripheral nociceptive input (and central sensitization) and peripheral neuroinflammatory response that could be related to severe postoperative pain and increased risk of persistent postoperative pain.[14–16] Unfortunately, this approach could be affected by some adverse effects such as risk of catheter displacement and lack of efficacy and infectious risk, patient activity reduction because of the use of pump to administer drugs, device malfunction, need to refill, and systemic toxicity.[17]

The development of local anesthetic platforms that provide prolonged local analgesia has classically been hindered by several pitfalls: inadequate duration of drug action, the appearance of systemic or organ-specific toxicity, and adverse local tissue reaction or inflammation.[17,18] Novel delivery platforms capable of providing such prolonged, postoperative analgesia without these limitations are valuable and necessary.

Carrier systems have emerged as an attractive alternative with their ability to promote all of the desirable characteristics for a local anesthetic, including: long duration of action, selectivity for sensory rather than motor nerve block, and reduction of systemic toxicity.[14,18–20] A variety of controlled-release formulations have been developed, such as polymeric microparticles (MPs)[21–24] or liposomes.[25–28] Such systems have extended the duration of nerve block to varying degrees ranging from hours to weeks, and some of the more basic, US FDA-approved formulations are now being adopted clinically. Only one such formulation is currently approved for clinical use [liposomal bupivacaine (BP), EXPAREL™], and its opioid-sparing effect is limited to 72 h.[27] Examples of MPs already FDA-approved

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for use include poly-e-caprolactone and poly(lactic-co-glycolic acid) (PLGA), but they can cause secondary inflammatory reactions—the nature of which appears strictly dependent on the type of material and size of the particles.[29–32] Large particle size (20 μm) promotes chronic inflammatory infiltrate with prominent foreign body giant cells, whereas smaller ones elicit a phagocytic and lymphocytic response only. Thus, the process of opsonization is one of the most important biological barriers to controlled drug delivery. The foreign body reaction and chronic inflammatory state can affect the final drug efficacy and release kinetics of different particle-loaded drugs.[30,33–35]

In this work, we aimed to develop an environmentally sensible delivery system capable of prolonged local anesthetic (BP) release, while avoiding foreign body reaction and cellular toxicity. We created PLGA MPs with a chitosan (Chi) coating because it offers several advantages, including: intrinsic antibacterial activity,[36] human biocompatibility (minimizes local inflammation and foreign body reaction), biodegradability,[37,38] enhanced hemostasis,[23,39] and ability to swell at acidic pH.[37] In the acidic extracellular matrix of inflamed tissue, Chi is fully protonated and when it swells, releases the entrapped drug. Moreover, we hypothesized that the incorporation of particles inside a thermoresponsive gel, pluronic F-127, will reduce the undesired local foreign body reaction, effectively cloaking the particles.[40,41]

MATERIALS AND METHODS

Bupivacaine hydrochloride, poly(vinyl alcohol), Rhodamine B, Chi (LMW), Pluronic F-127, and all other reagents used were of analytical grade, and were purchased from Sigma–Aldrich (St. Louis, Missouri). PLGA-ester-terminated (lactide–glycolide = 50:50) viscosity range 0.95–1.20 dl was purchased from LACTEL (Pelham, Alabama).

Preparation of PLGA–Chi–BP Particles

Bupivacaine-loaded PLGA–Chi MPs were prepared by a modified single emulsion method as mentioned in our previous studies.[42] Briefly, PLGA (50:50) was dissolved in dichloromethane (DCM) to form 10% (w/v) PLGA–DCM solution. Twenty milligrams of BP or 1 mg of Rhodamine B was added while sonicating the mixture. The organic phase of the mixture containing the drug was mixed with polyvinyl alcohol 2.5% (w/v) and (0.5%) Chi by vortex mixing and sonication. The resulting suspension was stirred with a magnetic stir bar for 4 h and the DCM was eliminated by evaporation. BP was also loaded on the outside Chi layer in order to synthesize PLGA–BP–Chi–BP MPs. Briefly, 10 mg of BP was loaded under acidic conditions (buffer acetate pH 5.5) in the Chi layer for 6 h at room temperature; the suspension was washed three times with ultrapure water by centrifugation at 4424g for 10 min, then the loaded MPs were freeze-dried and stored at −80°C for later use.

Characterization of PLGA MPs

Dynamic light scattering (DLS) was performed using a Zetasizer ZEN3600 (Malvern, Worcestershire, UK). For DLS, scattered light detection was measured at 90° to the incident beam (a 25 mW laser at 660 nm wavelength). MPs size distribution was measured using a Beckman Multisizer 3 Coulter Counter (Beckman Coulter, Brea, CA). Fourier transformed infrared (FT-IR) spectroscopy was performed by creating a pellet of 5% sample and 95% KBr (Sigma–Aldrich) by volume and analyzing absorbance of the pellet on a Nicolet 6700 FT-IR Spectrometer (ThermoFisher Scientific Inc, Walthman, MA). The spectra were reported after background subtraction, baseline correction, and binomial smoothing (11 points) using OMNIC software (ThermoFisher Scientific Inc, Walthman, MA). The morphology of the MPs was characterized by optical microscope (Nikon Eclipse TS 100, Nikon Instrument Inc., Melville, NY), fluorescent microscope (Nikon Eclipse TE 2000-E, Nikon Instrument Inc., Melville, NY), confocal laser microscope (Leica MD 6000, Leica Microsystem, Inc., Buffalo Groove, IL), and finally scanning electron microscope (SEM) (FEI Quanta 400 ESEM FEG, FEI, Hillsboro, OR) under a voltage of 5 KV. Samples were sputtered with platinum (5 nm) by a Plasma Sciences CrC-150 Sputtering System (Torr International, Inc., New Winsdor, NY) before SEM analysis.

Cytoxicity Analysis of PLGA–Chi Particles

The effect of empty PLGA–Chi MPs on the viability of cells was determined by an Alamar Blue assay.[43] Bone-marrow-derived mesenchymal stem cells (mBM-MSCs) from mouse were isolated as previously reported for other species,[44,45] and the absence of mycoplasma contamination was determined using the MycoAlert mycoplasma detection kit (Lonza, Rockland, Maine). The cells (10^5 cells/well) were cultured on 24-well plates with 500 μL of Dulbecco's modified Eagle's medium/well. The mBM-MSCs were treated with 50 μg/mL MPs and cellular viability was evaluated at days 1, 3, 5, and 7. Cellular metabolic activity was quantified via the AlamarBlue™ assay (Invitrogen, Carlsbad, CA). At each time point, culture medium was removed and replaced with 500 μL of complete medium with 10% AlamarBlue™ reagent and incubated for 2 h at 37°C, 5% CO_2. Absorbance was measured using a plate reader at wavelengths of 570 and 600 nm, taking into account the background absorbance of both the medium and reagent only (negative control). Cellular metabolic activity was correlated with the percentage reduction of AlamarBlue™ reagent by the cells, and calculated according to the manufacturer's protocol (www.biosource.com). All experiments were conducted in triplicate.

Overall Phagocytosis Assay

Murine peritoneal macrophages J774 (Lonza, Basel, Switzerland) were seeded in 12-well plates at a concentration of 2×10^5 cell/mL and were allowed to adhere for 24 h. MPs (Rhodamine labeled) were added to the cells at a concentration of 50 μg/mL (small and big PLGA–Chi, respectively) and incubated for 24 h at humidified 37°C, 5% CO_2. Moreover, same amount of cells were seeded on the 25% Pluronic F-127 mixed with 50 μg/mL of MPs. After 24 h, the culture medium of each sample in both experiments was collected and the concentration of inflammatory cytokines IL-6 and TNF-α produced by J774 cells was quantified by ELISA assay (R&D Systems). Cells were then scraped from the wells, washed three times with cold phosphate-buffered saline (PBS) to remove unattached particles and analyzed using flow cytometry. SEM was used to visualize cellular interaction with MPs. After allowing 24 h of cellular attachment to glass coverslips at 37°C, cells were fixed with 2.5% glutaraldehyde (Sigma–Aldrich), washed with increasing concentrations of ethanol (up to 100%), vacuum dried, and coated with palladium prior to SEM visualization (Hummer 6.2 Sputtering System; Anatech Ltd., Union City, California).