International Journal of Pharmaceutics 468 (2014) 152–157

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Extended duration local anesthetic agent in a rat paw model

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ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 7 April 2014
Accepted 8 April 2014
Available online 13 April 2014

Keywords:
Bupivacaine
Injectable local anesthetics
Polymer
Prolonged release

ABSTRACT

Encapsulated local anesthetics extend postoperative analgesic effect following site-directed nerve injection; potentially reducing postoperative complications. Our study aim was to investigate efficacy of our improved extended duration formulation – 15% bupivacaine in poly(DL-lactic acid co castor oil) 3:7 synthesized by ring opening polymerization. *In vitro*, around 70% of bupivacaine was released from the p (DLLA-CO) 3:7 after 10 days. A single injection of the optimal formulation of 15% bupivacaine-polymer or plain (0.5%) bupivacaine (control), was injected via a 22G needle beside the sciatic nerve of Sprague-Dawley rats under anesthesia; followed (in some animals) by a 1 cm longitudinal incision through the skin and fascia of the paw area. Behavioral tests for sensory and motor block assessment were done using Hargreave's hot plate score, von Frey filaments and rearing count. The 15% bupivacaine formulation significantly prolonged sensory block duration up to at least 48 h. Following surgery, motor block was observed for 48 h following administration of bupivacaine-polymer formulation and rearing was reduced (returning to baseline after 48 h). No significant differences in mechanical nociceptive response were observed. The optimized bupivacaine-polymer formulation prolonged duration of local anesthesia effect in our animal model up to at least 48 h.

1. Introduction

Surgical incision damages skin, fascia and muscles (Kawamata et al., 2002; Zahn et al., 2002); resulting in pain that is exacerbated by coughing, ambulation and mechanical stimulation. Use of currently available drugs for analgesia is often associated with side-effects and unreliable efficacy (Marcil et al., 2006). Previously, we described the synthesis of a polyester based castor oil and lactic acid by polycondensation, that prolonged the effect of directly injected bupivacaine from 8 to 48 h in mice (Sokolsky-Papkov et al., 2009a).

Biodegradable polyesters based on polylactide acid (PLA) are among the most frequently used polymers. Ring-opening polymerization (ROP) was recently described as a main polymerization method for polymer synthesis (Albertsson and Varma, 2003; Yu et al., 2005); facilitating polymerization under mild conditions to achieve high molecular weight polyesters (Jerome and Lecomte, 2008). This results in reduced factor variability for polymer production; ultimately increasing reliability and reducing costs.

The aim of the current study was to prepare an improved encapsulating agent p(DLLA:CO) 3:7 by ROP, and to demonstrate duration of action of this formulation using an *in vivo* model of incisional pain in a rat model.

2. Materials and methods

2.1. Materials

DL-lactide purasorb, Purac lot 0508000022, tin(II) 2-ethylhexanoate, Sigma Batch 035K0245 and castor oil Tamar Pharm BN4700801. Bupivacaine HCl USP 26 was purchased from Eurotrade, Commerce, S.L. Bupivacaine HCl 0.5% solution, Kamada, Israel. All solvents and salts were analytical grade from Aldrich or BioLab (Jerusalem, Israel).

2.2. Instrumentation

Bupivacaine concentrations in buffer solutions were determined as previously described (Shikanov et al., 2007; Sokolsky-Papkov et al., 2009a,b) using high-performance liquid chromatography (HPLC) with a C18 reverse phase column

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http://dx.doi.org/10.1016/j.ijpharm.2014.04.022
0378-5173/

(LichroCartR 250-4, LichrospherR 100, 5 mm). A mixture of 35% acetonitrile: 65% phosphate buffer 0.1 M pH 6.8 at a flow rate 2 mL/min was used as eluent and UV detection at 263 nm (injection volume 50 μL, run time 12 min).

2.3. Polymer synthesis

Poly(DL:lactic acid co castor oil) 3:7 – designed as p(DLLA-CO) 3:7 – was synthesized by ring opening polymerization. In brief, castor oil, DL-lactide and tin(II) 2-ethylhexanoate 1% (tin octoate ($Sn(Oct)_2$), in castor oil were reacted in a closed flask dried under nitrogen flow at 140 °C for 48 h. The resultant polymer was a clear liquid at room temperature with Mn 2200 and Mw 2300 Da as determined by gel permeation chromatography (GPC).

2.4. Preparation of formulation, improvement of the formulation, and in vitro *drug release*

Bupivacaine free base was prepared from bupivacaine hydrochloride by alkaline precipitation and filtration as previously described (Sokolsky-Papkov et al., 2009b). Bupivacaine free base (15%, w/w) was reduced to 0.25 mm particle size by sieving the powder through a 60 mesh sieve. The powder was incorporated by mixing the homogenic drug powder into the liquid polymer at room temperature to produce a viscous injectable liquid. This resulted in an improved formulation that was loaded into a 1 mL tuberculin syringe. The variations included in the formulation allowed easy passage through a 22G syringe without effort.

In vitro drug release studies were conducted by injecting 0.15 mL of the 15% bupivacaine-polymer formulation in a 50 mL of dissolution medium (phosphate buffered saline, PBS, pH 7.4) at 37 °C with constant shaking (100 rpm), where it formed a droplet in the buffer. The releasing medium was replaced periodically with fresh buffer solution and the bupivacaine concentration determined by HPLC as previously reported (Sokolsky-Papkov et al., 2009a,b). All experiments were done in triplicate.

2.5. Animals

The study received approval from the ethics committee of the Hebrew University Hadassah Medical School (National Institutes of Health approval number: OPRR-A01-5011) for performance of animal studies (ethics committee research number: MD-11-12672-4).

Male Sprague-Dawley (Hd/SD) rats ($n = 30$), approximately 250 g were housed with four in a cage with free access to food and water. The animal room was light cycled (12 h light, 12 h dark), and the temperature was 22 °C. Animals were weighed 24 h prior to the experiment and at 7 days post-experiment.

2.6. In vivo *model*

The animals were acclimated to behavioral testing 24 h prior to the experiment. All animal experimental procedures were carried out under deep anesthesia performed by i.p injection of ketamine and xylazine solution (80:20) to facilitate identification and injection of the formulation at the sciatic nerve (Wixson et al., 1987). The right (ipsilateral) leg received no drug. Animals received either single injection of 1 mL of the formulation (15% bupivacaine-polymer, $n = 12$) or 1 mL of 0.5% plain bupivacaine solution ($n = 12$). This was injected via a needle of 22G diameter directly close to the left sciatic nerve using a nerve stimulator (StimuplexR B.Braun Melsungen AG, Germany) at 0.2 mA and 1 Hz to identify the sciatic nerve.

After the sciatic nerve injection (still under anesthesia), we randomly selected 12 animals (6 rats treated with 15% bupivacaine-polymer formulation and 6 with plain bupivacaine) for the incisional model. We cleansed the left hindpaw with iodine solution and made a 1 cm longitudinal incision through the skin and fascia in the central area of the plantar (Brennan et al., 1996; Cameron et al., 2008) aspect, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. Following hemostasis through pressure, the skin was apposed with 2 mattress sutures of 5–0 nylon.

A control group ($n = 6$) received no sciatic nerve injection and no plantar incision (used for motor tests and some behavioural tests)

2.6.1. Behavioral testing

The testing room was illuminated with indirect white lighting. Behavioral tests began 24 h post-anesthesia; animals recovered in their cages. We observed spontaneous exploratory activity as surrogate measure of postoperative pain; animal behavior within the cages and changes to baseline were noted. These evaluations were done prior to sensory testing to minimize interference by external pain exacerbation.

2.6.1.1. Sensory block. The tests were performed using thermal hindpaw hyperalgesia (Hargreave's hot plate) and mechanical nociceptive response (von Frey filaments) to measure time to withdrawal of the tested leg.

2.6.1.2. Thermal hindpaw hyperalgesia. This was done for animals receiving sciatic nerve injection without paw incision. We placed the animal at rest with the paw in direct contact with the glass plate (Hargreaves et al., 1988). After 5 min acclimatization, we applied the center of an infrared radiant beam (standard heat temperature 50–52 °C) to the plantar surface of the hind paw. Withdrawal latency was measured (time to withdraw the hind paw from the glass hot plate) for alternating paws, allowing at least 5 min recovery between plate measurements. Each paw was tested 5 times – a total of 10 tests per animal at each evaluation. The results were expressed as the mean of the five withdrawal latencies (ds). Tests were performed at 24, 48, 72 and 96 h.

2.6.1.3. Mechanical nociception response. This response was evaluated using von Frey hairs (Touch Test™, U.S.A) expressed in grams in animals following plantar incision. Animals were placed on a metal mesh (22 × 32 cm) and acclimatized for 15 min. We stimulated the plantar surface of the paw using von Frey filaments of ascending force, applied (from underneath) through the opening of the metal mesh, until the animal withdrew the paw in response to the force used. The force ranged from 0.008 to a maximum of 26 g. For each force filament, the stimuli were repeated three times in both legs with an interval of 1 min between each stimulus. The threshold was determined as the lowest force evoking a withdrawal response of the stimuli (Marcil et al., 2006), (expressed in grams) at paw withdrawal: the lower the score, the more sensitive the paw. If no change in sensation was noted after three stimuli, the von Frey hair with the next force was used. If no response was obtained with the cutoff value of 26 g, the value recorded was >26 g. For this test, left legs were compared to right leg of the animals (control leg), since each leg respond independently to the stimuli.

2.6.1.4. Motor block. It was assessed in all animals as we described previously (Shikanov et al., 2007) using a four-point scale: 0 = loss of dorsiflexion, flexion of toes, and impairment of gait, 1 = toes and foot plantar flexed with no splaying ability, 2 = intact dorsiflexion of foot with impaired ability to splay toes when elevated by the tail, 3 = normal. In addition we measured proprioception (present or absent), grip (present or absent) and limp (present or absent) on a scale 0 = none, 1 = present. Rearing was recorded as the number of times the animal stood completely erect on its hind legs. Animals