Extended Release Local Anesthetic Agents in a Postoperative Arthritic Pain Model

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ABSTRACT: Local anesthetics play an important role in postoperative pain management in orthopedic joint procedures. The aim of this study was to determine the effect of an intraoperative extra-articular injection of poly(DL-lactic acid co castor oil 3:7), p(DLLA:CO) 3:7 loaded with 15% bupivacaine, for postoperative analgesia following knee arthroplasty. Prolonged release local anesthetic formulation was synthesized by mixing p(DLLA:CO) 3:7 with bupivacaine base. Under anesthesia, the knee joint of Sprague–Dawley rats was exposed, a hole drilled in the femoral trochlea. 0.2 mL of either 15% polymer–bupivacaine formulation or plain bupivacaine (control) was injected locally and compared with a nonsurgery control group. Mechanical hyperalgesia was determined by counting the vocalizations and leg withdrawal after joint squeezing. Behavioral assessments over a day postoperative period revealed a reduction in rearing and ambulation in an open-field apparatus in animals of both experimental groups compared with the nonsurgery control. The vocalizations during the hyperalgesia test increased compared with the control at 24 h. At 48 h, 3.667 ± 0.5138, $p = 0.0076$ vocalizations were recorded for the plain bupivacaine group versus 1.417 ± 0.5138, $p < 0.0001$ in the 15% polymer–bupivacaine formulation. Bupivacaine encapsulated in p(DLLA:CO) 3:7 extended the duration of the analgesia compared with plain drug in rats and could represent effective postoperative analgesic in orthopedic joint procedures. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:185–190, 2014

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INTRODUCTION

A major clinical concern is the pain resulting from musculoskeletal disorders. Orthopedic operations are among the most painful procedures, with cost implications when hospital discharge is delayed because of pain.[1,2] The development of analgesic treatment options for arthritic pain could be simulated using animal models.[3,4] Current analgesic therapies include nonsteroidal anti-inflammatory drugs and opioids, with their adverse effects such as suppression of platelet aggregation, gastrointestinal mucosa disruption,[5] nausea, vomiting, excessive sedation, and respiratory depression.[6,7] New treatments affecting the nociceptive process with no central side effects are a desirable goal.[8,9] For orthopedic procedures, instillation of intra-articular local anesthetics during arthroscopic procedures may be effective.[10–12] Assessment of the efficacy of such therapeutic agents can be performed using an animal arthritic model, measuring weight bearing, foot position, gait analysis, paw elevation time, and mechanical or heat sensitivity of the paw. Behavioral tests such as mechanical or heat sensitivity of the paw would be an indirect measure, as this is distant to the surgical site.[3,13,14] Novel biodegradable polymers based on fatty acids have been developed over the last decades.[15–17] We previously reported an effective bupivacaine poly(lactic co castor oil) formulation for sciatic block in mice. *In vitro* studies of the 15% bupivacaine poly(DL-lactic acid co castor oil 3:7), p(DLLA:CO) 3:7 polyester showed that approximately 65% of the loaded drug was released during the first week without burst effect during the first hours, resulting in an optimal formulation that extended the duration of the anesthesia in an animal nerve block model. Histopathological evaluation following injection of the formulation confirmed its biocompatibility.[18] The aim of the current study was to determine whether an intraoperative extra-articular injection of our p(DLLA:CO) 3:7 loaded with 15% bupivacaine, a local anesthetic, is efficacious following knee arthroplasty or not. This simple inexpensive method could provide an effective adjunct to improving postoperative recovery following knee surgery.

MATERIALS AND METHODS

All animal experiments were approved by 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).

Materials

DL- lactide purasorb, Purac lot 0508000022 (Gorinchem, The Netherlands); tin (II) 2-ethylhexanoate, Sigma batch 035K0245

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(Jerusalem, Israel); castor oil, Tamar Pharm BN4700801, (Jerusalem, Israel); Bupivacaine HCl USP 26, Eurotrade, Commerce, S.L; Bupivacaine HCl 0.5% solution, Kamada, (Jerusalem, Israel) . All solvents and salts were analytical grade from Aldrich or BioLab (Jerusalem, Israel).

Polymer Synthesis and Formulation

Poly(DL- lactic acid co castor oil) 3:7 was synthesized by ring opening polymerization. The resultant polymer was a clear liquid at room temperature with Mn 2200, Mw 2300 Da. Bupivacaine free base was prepared from bupivacaine hydrochloride by alkaline precipitation and filtration as previously described.[16] The free-base bupivacaine (15%, w/w) was incorporated by mixing the drug powder into the liquid polymer at room temperature to produce a viscous injectable liquid. The formulation was loaded into a 1-mL tuberculin syringe for easier application in the affected area.

Animals

A total of 28 male Hd/SD rats (Harlan, Israel) weighing approximately 250 g were housed up to four in a cage with free access to food and water in a Specific Pathogen Free unit, where all experiments were performed. The animals' room was light cycled (12 h light, 12 h dark), and the temperature was 22°C.

Surgical Procedure

Animals were weighed prior to surgery and 1 week postoperatively. Three groups were evaluated: (a) control group receiving no surgery or formulation infiltration, (b) treatment group receiving formulation based on p(DLLA:CO) 3:7 containing 15% bupivacaine, and (c) treatment group receiving plain bupivacaine HCl 0.5%. All experimental procedures were carried out under anesthesia, by i.p. injection of ketamine and xylazine (40–80 mg/kg BW IP and 5–10 mg/kg BW IP).[19] Animals were placed in supine position; the left knee was flexed and shaved. Under sterile conditions following disinfection by 70% ethanol, a central longitudinal skin incision approximately 1 cm was performed. The knee articular surfaces were exposed using a medial parapatellar approach. Using a 16G needle, a 2 mm hole was drilled in the trochlea of the femur, 2 mm above the tibio-femoral joint line (Fig. 1). Following surgery, the leg was straightened to allow the patellar tendon to return to midline and joint capsule was sutured with absorbable Vycril 4.0 sutures. The skin over the incision was sutured with 4.0 nylon sutures. While the animal was still under anesthesia, a 21G needle connected to the 1-mL syringe loaded with the respective formulation (15% polymer–bupivacaine formulation or plain bupivacaine 0.5%) was used to inject into the extra-articular knee joint. Each treatment group animal received a single injection (0.2 mL) of either 15% polymer–bupivacaine formulation or plain bupivacaine 0.5%. This injection was made into the anterior aspect of the knee 1 cm above the knee joint parallel to the medial border of the surgical stiches. The wound was covered with bacitracin unguentum to avoid contamination. The optimum injection site and method were determined in a previous pilot study, to ensure optimum placement of the drug formulation. Suturing was performed prior to drug injection to avoid leakage or loss of drug during the suturing phase. The animals were observed daily for sign of restlessness, wound infection, dehiscence, or hematoma that would exclude further study.

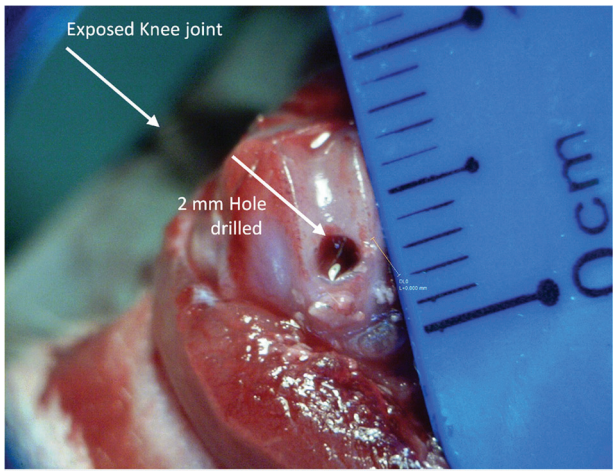

Figure 1. Rat arthritic model. Knee joint exposure with a 2-mm hole simulating open knee surgery. The hole depicted was made using a drill under a microscope for illustration purposes.

Observations

The same researchers performed all surgical procedures (D.I., L.G., C.F.W.). Two researchers, (D.I. and C.F.W.), performed animal behavioral evaluation. The observer counting the rearing and hyperalgesia, and performing squeezing and withdrawal assessments was blinded to the group allocations (C.F.W.).

Behavioral testing was conducted for baseline data on the day preceding surgery. The testing room was illuminated with indirect white lighting. Following knee surgery, spontaneous exploratory activity was measured by observing movement in the cages and changes in comparison to the control nonsurgery group were noted. All behavioral evaluations were performed before the hyperalgesia test to avoid any external pain exacerbation during the testing confounding the results. Changes in behavior were compared among the plain bupivacaine, the 15% polymer–bupivacaine formulation, and no-surgery control group.

Functional assessment of the inflamed rat paw knee joint was based on the observation of the animal placed in a single chamber (20 cm diameter) with clear plastic walls. Animals were placed individually and allowed time for habituation. During a 5-min evaluation period, rearing was recorded as the number of times the animal stood completely erect on its hind legs during this period (Fig. 2).

Ambulation test in an open field apparatus consisted of a plastic box ($37 \times 42 \times 20$ cm^3) with the floor divided by black lines to form rectangles (14×18 cm^2 each rectangle). Animals were gently placed in the middle of the field. The number of crossing squares (with all four paws) that the animal walked within a period of 3 min was recorded. Each animal was tested individually and following evaluation the working area was cleaned before placing the subsequent animal (Fig. 3).

Mechanical hyperalgesia was evaluated as a response to constant pressure developed while squeezing the knee junction in the medio-lateral direction and vocalizations were counted. In addition, leg withdrawal was noted when pressure was applied using a modification to the Randall–Selitto method[20–22]; while holding the animal in the palm of the hand of one researcher (D.I.), the thigh was fixed between the thumb and forefinger, and squeezed firmly (constant pressure applied for 10 s) by the

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