Pharmaceutical Research, Vol. 26, No. 1, January 2009 (© 2008)
DOI: 10.1007/s11095-008-9699-8

Research Paper

Prolonged Local Anesthetic Action Through Slow Release from Poly (Lactic Acid Co Castor Oil)

Marina Sokolsky-Papkov,[1] Ludmila Golovanevski,[2] Abraham J. Domb,[1] and Carolyn F. Weiniger[2,3]

Received May 11, 2008; accepted July 25, 2008; published online August 22, 2008

Purpose. To evaluate a new formulation of bupivacaine loaded in an injectable fatty acid based biodegradable polymer poly(lactic acid co castor oil) in prolonging motor and sensory block when injected locally.
Materials and methods. The polyesters were synthesized from DL-lactic acid and castor oil with feed ratio of 4:6 and 3:7 *w/w*. Bupivacaine was dispersed in poly(fatty ester) liquid and tested for drug release *in vitro*. The polymer p(DLLA:CO) 3:7 loaded with 10% bupivacaine was injected through a 22G needle close to the sciatic nerve of ICR mice and the duration of sensory and motor nerve blockade was measured.
Results. The DL-lactic acid co castor oil p(DLLA:CO) 3:7 released 65% of the incorporated bupivacaine during 1 week *in vitro*. Single injection of 10% bupivacaine loaded into this polymer caused motor block that lasted 24 h and sensory block that lasted 48 h.
Conclusion. Previously we developed a ricinoleic acid based polymer with incorporated bupivacaine which prolonged anesthesia to 30 h. The new polymer poly(lactic acid co castor oil) 3:7 provides slow release of effective doses of the incorporated local anesthetic agent and prolongs anesthesia to 48 h.

KEY WORDS: analgesia; local anesthetic; long-acting; mice; polymer–bupivacaine.

INTRODUCTION

Recent advances in experimental drug delivery permit huge doses of analgesic agent to be administered with a release which is slow, constant, effective and non-toxic (1–5). Such agents should improve the management of postoperative pain which is a major problem for the healing process after surgery. Direct injection of local anesthetics is the most site-directed and effective analgesic modality for the management of postoperative pain. Local anesthetics reversibly block nerve conduction near their site of application thus producing temporary loss of sensation over a specific area of the body (1). Previously we described a polyester-anhydride based on ricinoleic acid which prolongs the effect of directly injected bupivacaine from 8 to 30 h. However the *in vitro* release had promised longer *in vivo* efficacy (6). Anhydride bonds are sensitive to hydrolysis, resulting in quick degradation and therefore drug release. Therefore the concept behind the development of the new polymer was to reduce hydrolysis and to create a more hydrophobic polymer carrier which could increase the degradation time of the polymer and prolong the drug release period for bupivacaine. The current study describes the development of polyesters based on castor oil for prolonging the efficacy of bupivacaine when injected directly.

[1] Department of Medicinal Chemistry and Natural Products, School of Pharmacy—Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.
[2] Department of Anesthesiology and Critical Care Medicine, Hadassah Hebrew University Medical Center, Ein Kerem, Jerusalem POB 12000, Israel.
[3] To whom correspondence should be addressed. (e-mail: carolyn fweiniger@gmail.com)

ABBREVIATIONS: BFB, bupivacaine free base; CO, castor oil; DDW, double distilled water; DSC, differential scanning calorimetry; GPC, gel permeation chromatography; HPLC, high-performance liquid chromatography; ICH, International Conference on Harmonization; IR, infrared spectroscopy; LOD, The limit of detection; LOQ, The limit of quantification; Mn, number average molecular weight; Mw, weight average molecular weight; NMR, nuclear magnetic resonance spectroscopy; PLA, polylactic acid; p(DLLA:CO), poly(DL-lactic acid-co-castor oil).

MATERIALS AND METHODS

Materials

Castor oil European Pharmacopoeia (Eur Ph) was obtained from Florish (Haifa, Israel). DL lactic acid (DLLA) was purchased from J. T. Baker (Deventer, The Netherlands).

Bupivacaine HCl USP 26 was purchased from Eurotrade, Commerce, S.L.

$CDCl_3$, for NMR, was purchased from Sigma-Aldrich (Rehovot, Israel). All solvents and salts were analytical grade from Aldrich or Biolab (Jerusalem, Israel).

Methods

Polyester Synthesis

Poly(DL lactic acid co castor oil) 4:6 and 3:7 designated as p(DLLA:CO) 4:6 and p(DLLA:CO) 3:7 were prepared using racemic mixture (DL) lactic acid. The polyesters were synthesized using one neck reaction vessel equipped with a mechanical stirrer on an oil heating bath. Lactic acid and castor oil in appropriate weight ratio were added into the vessel. The catalyst, H_3PO_4 0.5% *w*/*w*, was added to the reaction mixture prior to polymerization. During esterification, the temperature was slowly increased up to 180°C and the mixture was stirred for 1.5 h under constant N_2 flow. The reaction vessel was connected to a pump and the reaction was continued under vacuum (15 mbar) for 24 h. The polymers were analyzed by IR, NMR, GPC and viscometer.

Molecular weight of the polymer was estimated on a gel permeation chromatography (GPC) system consisting of a Waters 1515 Isocratic HPLC Pump, with 2410 Refractive Index detector (RI) (Waters, MA), a Rheodyne (Coatati, CA) injection valve with a 20 μL loop. Samples were eluted with chloroform through a linear Styrogel column, 500 Å-pore size (Waters, MA) at a flow rate of 1 mL/min. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA) with a molecular weight range of 500 to 12,000 using BREEZE 3.20 version, copyright 2000.

Infrared spectra were performed on monomer and polymer samples cast on NaCl plates from dichloromethane solution on Bruker (Vector 22 System FT-IR). UV spectra were taken on a Kontron Instruments Uvicon model 930 (Msscientific, Berlin, Germany), Waters corporation computer program. 1H NMR spectra (in $CDCl_3$) was recorded on a Varian 300 spectrometer using TMS as internal standard (Varian Inc., Palo Alto, CA, USA).

Viscosity of polymers was measured using a Brookfield LVDV-III programmable viscometer coupled to a temperature-controlling unit. Cylindrical spindle LV4 was used. Temperature sensitivity test was performed starting at temperature of 55°C and down to room temperature (25°C) by applying constant rotational speed. Detection of rheological behavior was performed by measuring shear stress and/or viscosity at different shear rates, starting at 21 s^{-1} and up to 41.8 s^{-1} depending on the polymer.

^{1}H nuclear magnetic resonance spectroscopy: (RA-Ricinoleic acid, LA-lactic acid, GL-glycerol),$CDCl_3$, p(DLLA:CO), δ): 5.45–5.30 (2H, m, C9–10, -CH=CH-, RA), 5.20–5.02 (1H, q, CH-CH3, LA), 4.94- 4.86 (1H, m, C12 HC–O–, RA), 4.41–4.21(4H, m, CH, GL) 2.38–2.24 (2H, m, C2–CH2, and 2H, m, C11–CH2), 2.01 (2H, m, C8–CH2), 1.68–1.50 (2H, m, C3–CH2, 2H, m, C13–CH2, and 3H, d, –CH3, *LA*), 1.34–1.25 (16H, m, C4–7 and C14–17), and 0.868 (3H, t, C18–CH3) ppm.

Preparation of Free Base of Bupivacaine

Bupivacaine free base (BFB) was prepared from bupivacaine hydrochloride by alkaline precipitation and filtration. Briefly, 10 g bupivacaine HCl was dissolved in 300 ml double distilled water (DDW). Solution of $NaHCO_3$ (2.6 g in 50 ml DDW) was slowly added. The mixture was allowed to stand for 10 min. The mixture was extracted with 300 ml of dichloromethane. The extraction was repeated three times. The dichloromethane fraction was dried over MgSO4, filtered and evaporated to yield clear oil. The BFB was dissolved in 40 ml ethanol and purified by precipitation into DDW. The precipitate was filtered, dried under vacuum over night, (m.p. 107–8°C), yield 89%.

Preparation of Formulation and In vitro Drug Release

The BFB (7.5 and 10% *w*/*w*) was incorporated by mixing the drug powder into the liquid polymer at room temperature to produce a viscous injectable liquid. This formulation was loaded into a 1 ml tuberculin syringe. *In vitro* drug release studies were conducted by injecting 100 or 150 μl of the bupivacaine–polymer formulation in a 50 ml of dissolution medium (phosphate buffer 0.1 M, pH 7.4) at 37°C with constant shaking (100 rpm), where it forms droplet gels in the buffer. The BFB solubility in buffer phosphate is 0.4 mg/ml (determined in our laboratory). A large volume of dissolution medium was used in order to simulate the *in vivo* sink condition so that the concentration of bupivacaine never reached more than 10% of its maximum solubility (7). The releasing medium was replaced periodically with fresh buffer solution. All experiments were done in triplicate. Bupivacaine concentrations in buffer solutions were determined by high-performance liquid chromatography (HPLC) using a C18 reverse phase column (LichroCartR 250-4, LichrospherR 100, 5 μm). A mixture of 65% acetonitrile: 35% Buffer Phosphate 0.01 M pH 6.8 at a flow rate 2 ml/min was used as eluent and UV detection at 263 nm (injection volume 100 μl, run time 12 min).

Validation and Calibration

The validity of the analytical procedure was established through a study of specificity, linearity and accuracy according to the compliance criteria laid down in the ICH guidelines (8). The linearity of the analytical procedure was evaluated by plotting the detector response (peak area) against analyte concentration. Linear regression analysis was applied to calculate the slope, intercept and linear correlation coefficient (R^2). The accuracy was established by quantitative determination of the bupivacaine amount in quality control samples and was expressed as percent recovery by the assay of a known amount of analyte in the samples (8). The limit of detection (LOD) was calculated as signal-to-noise ratio of 3:1, and the limit of the quantification (LOQ) was determined as signal-to-noise ratio of 10:1 (8). Calibration curves bupivacaine in release medium were obtained by programmed injection of different aliquots (10–100 μl) of a standard solution with increments of 10 μl. The concentration of the standard solution was 10 μg/ml.

In vitro Hydrolytic Degradation of the Polymer

Polymer degradation studies were performed for p (DLLA:CO) 3:7. Samples (200 mg) of polymer were incubated in 50 mL buffer phosphate solution pH 7.4, 0.1 M at 37°C, with orbital shaking (100 rpm). The buffer solution was replaced periodically with fresh buffer solution. All experi-

ments were done in duplicate. At each time point, total of 1.5 mL of the buffer solution was extracted with 2 mL of CHCl3, dried on MgSO4, filtered, and concentrated to 250 μL. At the same time points, samples were taken from the polymers, rinsed with water, and lyophilized. The samples were analyzed by GPC. The degradation of polymers was followed by weight loss and GPC analysis for two months. The composition of the degraded polymers at different time points was determined by ^{1}H NMR. Typically, samples of 20 mg were diluted with 1 mL of CDCl3 and immediately analyzed. Lactic acid concentrations in buffer solutions were determined by high-performance liquid chromatography (HPLC) using a C18 reverse phase column (LichroCartR 250-4, LichrospherR 100, 5 μm). A mixture of 0.1% H_3PO_4 in double-distilled water (DDW) at a flow rate of 1 mL/min was used as eluent and UV detection at 210 nm. The calibration curves were plot in the range 0.01–0.1 mg/mL (R^2 = 0.9998).

In vivo Efficacy of the Formulation

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_83.02–4). The research adhered to the "Principles of Laboratory Animal Care" (NIH publication no. 85–23, revised in 1985). Female ICR mice weighing approximately 30 g were housed ten 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.

The animals were anesthetized with volatile anesthetic Isoflurane solution to facilitate identification and injection of the formulation at the sciatic nerve. Two formulations containing 10% bupivacaine (approximately 333 mg/kg) were injected; p(DLLA:CO)4:6 and p(DLLA:CO)3:7 based formulation. The sciatic nerve was identified using a nerve stimulator (StimuplexR B.Braun Melsungen AG, Germany) at 0.2 mA and 1 Hz *via* a needle of 22G diameter (6). Each animal received a single injection (0.1 ml) of the 10% bupivacaine–polymer solution in one leg and 0.1 ml 0.9% saline solution or polymer without the drug (blank polymer) on the contralateral side. Efficacy tests included both sensory and motor evaluation. Sensory tests were performed using the Hargreave's hot plate as described previously to measure time to withdrawal of the tested leg (9). Four groups of five animals were used at 4, 24, 48 and 53 h. Each leg was tested 5 times at one single time period, thus a total of ten tests for each animal. Motor block was assessed 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, proprioception (present or absent), grip (present or absent) and limp (present or absent) were measured on a scale 0 = none, 1 = present.

Maximal Tolerated Dose (MTD) Determination

The animals were anesthetized with volatile anesthetic Isoflurane solution to facilitate identification of the sciatic nerve and injection of the formulation at the sciatic nerve. Five groups, each 4 mice (total 20) were injected with different volumes (100, 110, 120, 140 and 160 cc) of p (DLLACO) 3:7 10% formulation were injected. Mice survival was monitored 3 and 24 h post injection.

Statistical Analysis

Data was analyzed to delineate a statistical difference between the drug (polymer–bupivacaine) and the control group (normal saline). Time to leg withdrawal on the hot plate (sensory blockade) was analyzed using a mixed model analysis of variance. The primary outcome was whether drug affected the time to withdrawal. Drug, experiment, and hour were considered as fixed effects, and animal (nested within experiment and hour) was considered to be a random effect. SAS Proc Mixed (Version 8.02) as used to perform the analyses. Motor response variables grip, splay, and proprioception were all binary in nature. Limp was not binary (values are 0, 1, 2, 3) thus it was dichotomized by choosing a cut off value. Since there was no pre-specified cut off value, all three possible cut-off were used and the study hypothesis was evaluated. The results were very similar thus only one of the analyses is presented (0 = 0, 1, 2 and 1 = 3 (no limp). The question of interest was whether drug affected these variables. A composite variable (Y) was created by summing these variables for each observation. Response variable Y was analyzed using a mixed model analysis of variance, with drug and hour defined as fixed effects and animal defined as a random effect. A p value < 0.05 is considered significant.

RESULTS

Polyester Synthesis and Characterization

Two new polymers, p(DLLA:CO) 4:6 and 3:7, were synthesized in order to prepare polymer with desired viscosity values. The polymers were prepared by direct polycondensation of DL-Lactic acid and castor oil (Fig. 1). p(DLLA:CO) 4:6 and 3:7 are a yellow liquid. The polymers have molecular weights Mw of 2,800 and 2,300 and Mn of 2,900 and 2,300 respectively. The polymers are summarized in Table I. The polymer possesses typical IR absorption at 1748 cm^{-}1, which corresponds to the ester carbonyl stretching bands. ^{1}H NMR

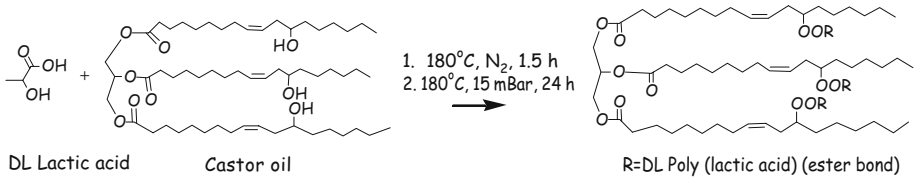


Fig. 1. Structure of poly(lactic acid co castor oil) synthesized by polycondensation.

Table I. Summary of Copolymers Used in the Study

Lactic acid/castor oil load	Calculated lactic[a] acid/castor oil ratio	Specific optical[b] rotation $[R]^{20}_{d}$	Mn/Mw[c]	Viscosity at room[d] temperature (cP)	Viscosity at[d] 37°C[c] (cP)
40:60	29:71	+12.48	Mn=2,800 Mw=2,100	3,400	900
30:70	22:78	+13.05	Mn=2,900 Mw=2,300	1,700	500

[a] Castor oil/lactic acid wt/wt ratio as calculated from ^{1}H NMR (by the integration ratio of the peaks at 5.15 ppm (one proton of PLA) and the peaks at 4.87 ppm one proton (C12) of RA).
[b] Optical rotations of polymers determined by an Optical Activity LTD polarimeter in a 20 mg/mL polymer solution in $CHCl_3$.
[c] Molecular weight determined by gel permeation chromatography
[d] Viscosity of the copolymers measured using a Brookfield LVDV-III programmable viscometer coupled to a temperature-controlling unit at 25°C.

spectra of the polymers fit their composition. All the ^{1}H NMR spectra lacked a peak at 3.64 ppm (m, 1H CH_2–CHOH–CH_2, ricinoleic acid of castor oil), and this indicated that there was no free alcohol of RA in the final product. The copolymer composition was verified and calculated from ^{1}H NMR by the integration ratio of the peaks at 5.15 ppm (one proton of PLA) and the peaks at 4.87 ppm 2.7 protons (C12) of RA in castor oil. The monomer composition of the final polymers correlates to the feed ratio of the monomers. The polymers are completely amorphous as confirmed by DSC.

The polymer behaves as Newtonian fluid, since its viscosity is not affected by shear rate applied (Fig. 2). Also the shear rate/ shear stress correlation was evaluated both at room temperature and 37°C. The polymer shows constant shear rate/shear stress behavior, confirming its properties as Newtonian fluid (Fig. 3). The p(DLLA:CO) 4:6 and 3:7 have viscosity of 3,400 and 1,700 respectively at room temperature, suggesting both polymers as potentially suitable carriers of bupivacaine.

In Vitro Drug Release

Bupivacaine was incorporated in the polymer without affecting the polymer molecular weight (Fig. 4). Bupivacaine free base (BFB) is easily soluble in p(DLLA:CO) 4:6 and 3:7 up to 20% *w/w* BFB. All the formulations were injectable through the 22G needle *in vitro*. The rate of bupivacaine free base release from p(DLLA:CO) is shown in Fig. 5. Total drug release from all p(DLLA:CO) formulations was quite similar with about 60% of the incorporated drug released in 7 days. However, the p(DLLA:CO) 4:6 released the drug in constant rate (almost first order release) for all 7 days. This release profile did not show rapid drug release in first 24 h (burst effect). The *in vitro* release from p(DLLA:CO) 4:6 in first week was slower then the release from previously reported polymer (6). For p(DLLA:CO) 3:7 the release profile was different. The total drug release from p(DLLA:CO) 3:7 was similar for each concentration of bupivacaine tested. Approximately 50% bupivacaine was released after 2 days and 60% of the drug released in 7 days. The sample size did not affect the release profiles from each formulation. The 7.5% formulation had the highest "burst effect" and released 50% of the bupivacaine in 7 h, with the 10% formulation releasing 35% in 7 hours. Less pronounced burst release effect, which is important in order not to create bupivacaine toxicity *in vivo*, makes 10% formulation of p(DLLA:CO) 4:6 and 3:7 more suitable candidates for *in vivo* efficacy evaluation.

Hydrolytic Degradation of the Polymer

Hydrolysis of the p(DLLA:CO) 3:7 was monitored by weight loss of the specimens (Fig. 6) and changes in polymer molecular weight (Fig. 7). Lactic acid release was also monitored and the remaining polymer was analyzed by ^{1}H NMR and IR. The p(DLLA:CO) 3:7 formulation lost 20% of

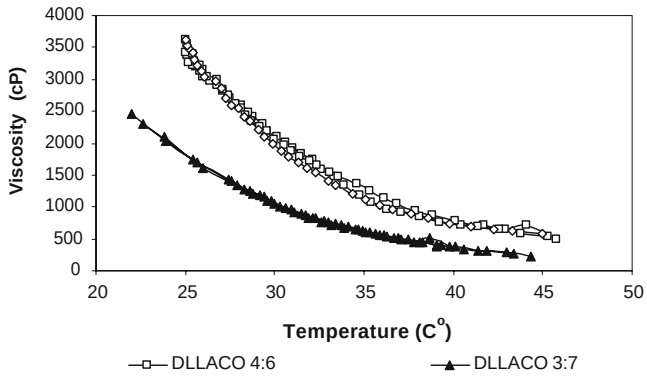


Fig. 2. Viscosity of p(DLLA:CO)4:6 and p(DLLA:CO)3:7 as a function of temperature at different shear rates (the shear rate applied is shown in the brackets). Polyesters were heated to 60°C and viscosity was measured using Brookfield LVDV-III programmable viscometer coupled to a temperature-controlling unit during polyester cooling. Cylindrical spindles were used. All tests were performed in triplicates.

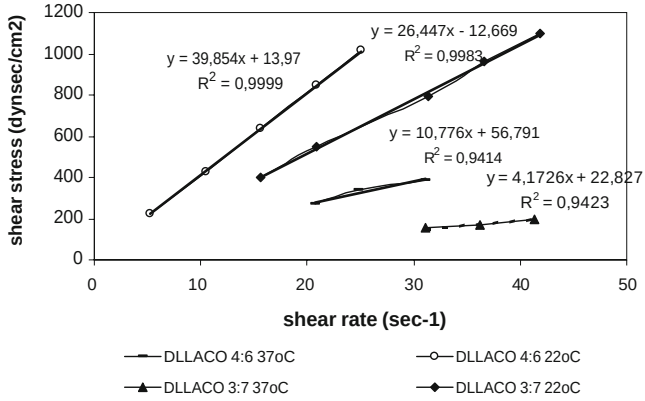
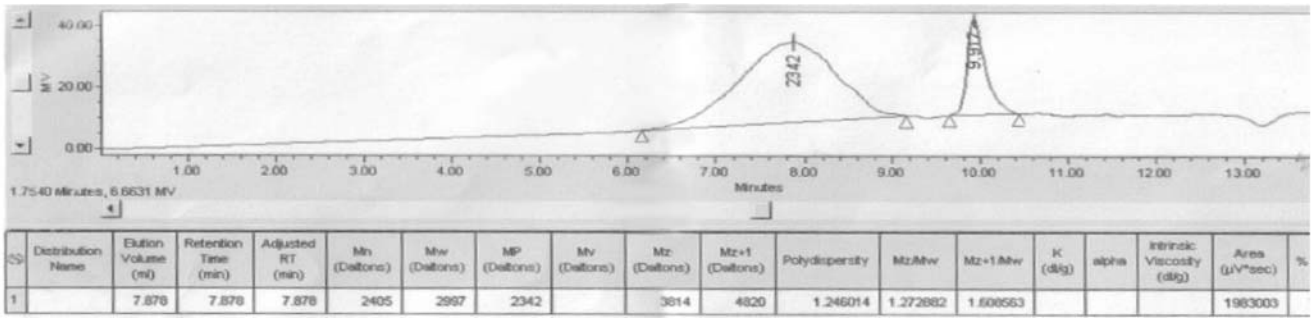


Fig. 3. Relationship of shear rate/shear stress of p(DLLA:CO)4:6 and p(DLLA:CO)3:7. Measurements were performed at 23°C using Brookfield LVDV-III programmable viscometer coupled to a temperature-controlling unit during polyester cooling. Cylindrical spindles were used. All tests were performed in triplicates.



	Distribution Name	Elution Volume (ml)	Retention Time (min)	Adjusted RT (min)	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mv (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw	K (dl/g)	alpha	Intrinsic Viscosity (dl/g)	Area (µV*sec)	%
1		7.878	7.878	7.878	2405	2997	2342		3814	4820	1.246014	1.272882	1.608563				1983003	

Fig. 4. The polymer–bupivacaine formulation as analyzed by GPC.

sample weight during 11 weeks (Fig. 4). The polymer lost about 20% of the weight in the first week. After 1 week polymer sample weight remained almost constant. The molecular weight (Mn and Mw) loss was monitored by GPC (Fig. 6). Mn of p(DLLA:CO) 3:7 decreased from 2,300 to 1,900 in 11 weeks of incubation. In the first 3 weeks a 10% decrease in Mn was seen. No significant change in Mn was observed until week 8. After 8 weeks, additional 10% decrease in Mn was observed. With regard to Mw, during the first week of incubation the molecular weight decreased from 2900 to 2600, remained unchanged for additional week and was followed by 1st order decrease until week 6. After 6 weeks, Mw reached 2,100 Da and remained constant until the end of the experiment. The lactic acid release showed that less of 1% was released during 11 weeks of hydrolysis. The ^{1}H NMR and IR spectrum of the remaining polymer showed no changes compared to original polymer. The probable explanation is that the decrease in polymer sample weight results from dispersion of the polymer in the release medium followed by the removal from the test tube and not a degradation of polymer chains.

In Vivo Efficacy

Formulation Selection

The p(DLLA:CO) 4:6 based formulation demonstrated a good *in vitro* release profile. The ability to inject this formulation was evaluated *in vitro* using the 22G administration needle and no potential problems were detected. However, the formulation was difficult to administer through the 22G needle *in vivo*. Therefore despite promising *in vitro* release pattern, the p(DLLA:CO) 4:6 based formulation was discarded. The experiments were continued with the p(DLLA:CO) 3:7 formulation only.

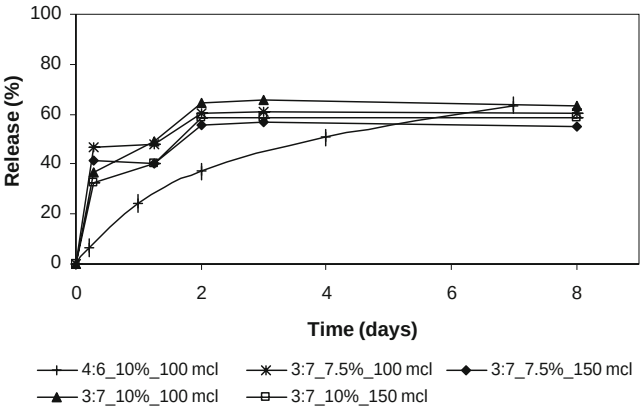


Fig. 5. *In vitro* release of Bupivacaine free base from p(DLLA:CO) 4:6 (10% *w*/*w*) and p(DLLA:CO) 3:7 (7.5 and 10% *w*/*w*) Bupivacaine release was conducted in a 0.1 M phosphate buffer (pH 7.4) at 37°C. Bupivacaine free base content in the releasing medium was determined by HPLC.

Sensory Tests

The 10% bupivacaine–polymer formulation significantly increased the time to withdrawal of leg using the Hargreaves hot plate at 4 h post-injection (64.4 ± 11.3 *versus* 48.7 ± 5.68 for drug *versus* control, the two-tailed $P < 0.0025$) (Fig. 8). 24 h post injection the time to reaction of the anesthetized leg was 61.1 ± 7.3 compared to 34.6 ± 3.44 in the control ($p < 0.0001$). At 48 h post injection the time to reaction of the anesthetized leg was 57.41 ± 7.1 compared to 38 ± 3.35 of the control ($p < 0.0001$). At 53 h post injection no significant difference in the time to withdrawal was observed indicating full recovery of the study nerve. Units of measurement of withdrawal of leg are 1/10 of a second. Five mice received blank polymer. Injection of blank polymer did not prolong the time to withdrawal.

Motor Tests

The bupivacaine slow release formulation showed significant levels of motor blockade during 24 h post-injection (Fig. 9). By 48 h the motor function was normal. The injection of blank polymer did not cause any impairment in motor function.

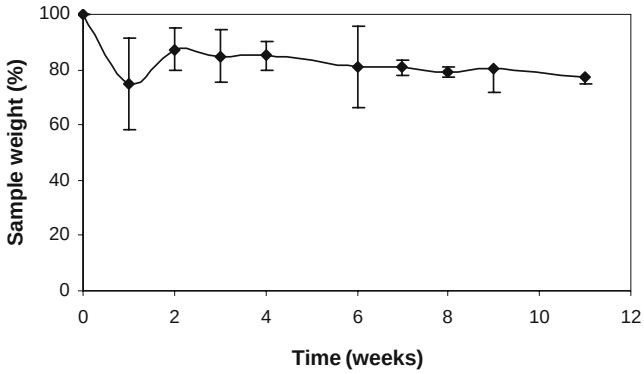


Fig. 6. Hydrolysis of p(DLLA:CO)3:7 monitored by specimen mass loss. Hydrolysis was conducted in 0.1 M phosphate buffer (pH 7.4) at 37°C.

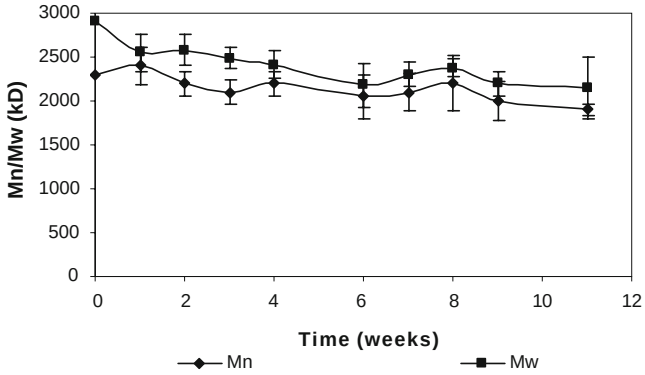


Fig. 7. Hydrolysis of p(DLLA:CO)3:7 monitored by the Mn loss, determined by gel permeation chromatography. Hydrolysis was conducted in 0.1 M phosphate buffer (pH 7.4) at 37°C.

Maximal Tolerated Dose

To establish the tolerated dose mice were injected with different volumes of 10% formulation. The survival was monitored up to 24 h post injection (Fig. 10). Even slight increase in dose was significant. At 110 cc group only 1 mouse survived 24 h post injection. Similar results were shown with mice received 120 and 140 cc (2 and 1 survived mice respectively). However, when the administrated dose was increased to 160 cc the toxicity decreased dramatically as three mice survived 24 h post injection.

DISCUSSION

In vivo evaluation demonstrated prolonged motor and sensory anesthesia with the formulation prepared from p (DLLA:CO)3:7compared with the baseline reference. We previously described the use of poly(sebacic co ricinoleic acid) which prolonged both motor and sensory block provided by bupivacaine to 30 hours however the duration of anesthesia was shorter than expected. The new castor oil based formulation prolonged sensory anesthesia to 48 h.

The polymer–drug formulation is a continuous matrix and the local anesthetic drug is dispersed throughout the polymer. Drug release and degradation rate from the carrier

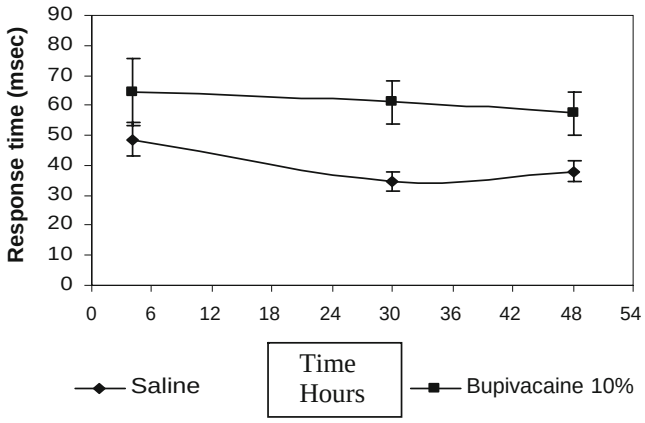


Fig. 8. Graph of withdrawal latency after administration of polymer formulation containing 10% *w/w* bupivacaine (*square*) or normal saline (*rhomb*). The data is represented as means±SD (units are 1/10 of a second). In all presented time points $p<0.0001$ significance in comparison to normal saline.

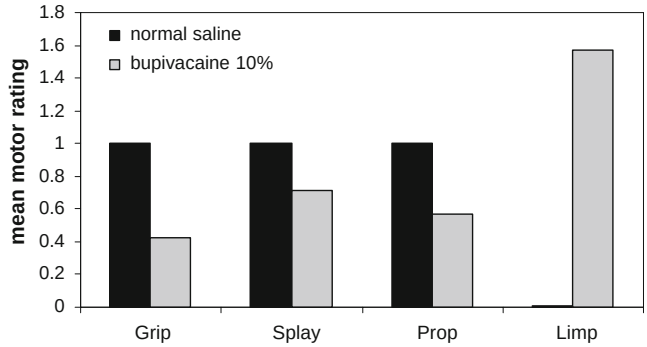


Fig. 9. Graph of motor block produced by administration of polymer formulation containing 10% *w/w* bupivacaine (*gray*) or normal saline (*black*). Grip, proprioception and limp (present or absent) were measured on a scale 0=none, 1=present. Splay was assessed according to a four-point scale: 4—normal, 3—intact dorsiflexion of foot with impaired ability to splay toes when elevated by the tail, 2—toes and foot plantar flexed with no splaying ability, 1—loss of dorsiflexion, flexion of toes, and impairment of gait (4). Using the Bonferroni adjustment for multiple comparisons, differences between times 4 and 24, 24 and 30 were examined. Only the 4–24 comparison was statistically significant at the Bonferonni-adjusted level ($p=0.0019$). There was no significance to a difference at 30 h.

can be manipulated by selecting polymer structure and bond type in the polymer chain. The current study describes two modifications of a previous polymer poly(ester-alhydride) prepared from two fatty acids (sebacic and ricinoleic) which was used as drug carrier to prolong the effect of bupivacaine (6). First, the polymer type was changed to polyester. Ester bonds are less sensitive than anhydride bonds to hydrolysis. The polyester is expected to have longer degradation periods compared to poly(ester-anhydride), resulting in prolonged duration of release of the incorporated drug (10,11). Second, the polymer composition was changed in order to increase the polymer hydrophobicity. Although lactic acid is more hydrophilic compared to sebacic acid, castor oil is more hydrophobic than ricinoleic acid. Drug release from the polymer is a function of water penetration into the polymer matrix and penetration of the drug out of the polymer. Increase in polymer matrix hydrophobicity slows water penetration into the polymer and increases the interactions between the polymer and the drug, hydrophobic bupivacaine (free base).

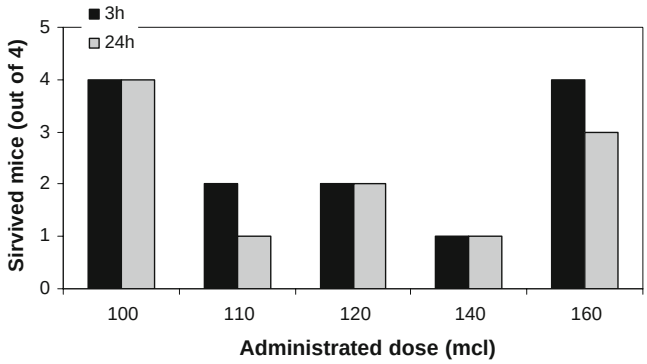


Fig. 10. Maximal tolerated dose (MTX). MTX was evaluated by injection of increasing volumes of 10% drug–polymer formulation and monitoring of animal survival at 3 h (black) and 24 h (gray) post injection.

Thus the drug forms stronger hydrophobic interactions with the polymer, contributing to slower release of the drug and prolonged bupivacaine drug efficacy.

It was expected that increase in castor oil content of the polymer will increase drug retention, resulting in slower release rates. However for all tested drug loads and sample sizes the release from p(DLLA:CO) 3:7 was faster compared to p(DLLA:CO) 4:6. The lower viscosity of p(DLLA:CO) 3:7 causes higher dispersion of the formulation in the test tube, increases the buffer-formulation contact area which results in more appropriate drug release. The maximal tolerated dose evaluations demonstrated indirect correlation between the toxicity and the dose administered. The mortality rate was not linear to injected dose. Then maximal volume (160 cc) was injected the mortality rate decreased (three out of four mice survived 24 h post injection) probably because larger volume of injected formulation forms a tight implant reducing the water penetration and drug diffusion.

Polymer viscosity was an important characteristic in polymer selection due to the direct administration of polymer–drug formulation *via* a 22 G nerve stimulator needle. However the viscosity of the polymer should be high enough to prevent polymer dispersion following injection. Bupivacaine incorporation into the polymer will increase the overall viscosity. The previously used poly(sebacic co ricinoleic acid) had viscosity in the range of 1,000 cP at room temperature and was easily injectable thus this formed the basis for our estimation of desired viscosity (12). Previous studies of poly (lactic acid co castor oil) showed that the viscosity of polymer synthesized from L-lactic acid is higher compared to polymer prepared from DL lactic acid. This was true to all tested polymer compositions (13). Also, the polymer viscosity decreases as castor oil ratio increases. However, previously prepared p(DLLA:CO) 5:5 had viscosity of 6600 cP at room temperature, considerably higher then the desired limit (13). By combination of these facts it was decided to focus on DL-lactic acid as monomer and prepare polymers with different lactic acid:castor oil ratio to achieve desired viscosity range.

The administration site *in vivo* is surrounded by muscle tissue and has no natural void. Therefore following injection, the resistance of tissues surrounding the nerve injection site caused plugs of polymer–bupivacaine to form in both the needle and attached injection tube when p(DLLA:CO)4:6 was administered. This was considered to be due to the higher viscosity of the formulation. Theoretically it is possible to preheat the formulation to reduce its viscosity. However, heating the formulation may alter the dispersion of the drug in the polymer matrix resulting in non-homogeneous drug load. In addition, the formulation will cool down during administration. During this cooling process the drug may precipitate and aggregate in the polymer matrix, and this also may contribute to a non-homogeneous drug load. Ease of formulation administration was considered to be a development aim. Since the p(DLLA:CO) 4:6 was impossible to inject after warming, or when frozen and defrosted, it was felt that to continue to perform animal experiments with this formulation, which was unreliable in the manufacturing stage, was not appropriate. No such problems were observed when p(DLLA:CO) 3:7 was administered as the formulation is easily injectable at room temperature *in vivo*.

The aim of the *in vivo* studies was to measure the duration of anesthesia provided by the bupivacaine–polymer formulation. The reference was injection of a passive solution (normal saline) to provide a non-anesthetized leg in the same animal. Anesthesia was present in the formulation leg when the withdrawal time was significantly longer than the reference leg of the same animal. Recovery of anesthesia occurred when the withdrawal time was similar to the baseline found in the same animal's reference leg. Single injection of plain bupivacaine alone has been previously examined and the duration of action found to be considerably shorter than that seen in continuous slow release formulations (5). The aim of the current *in vivo* study was to measure the actual duration of anesthesia provided by the new bupivacaine formulation with reference to the time to withdrawal in the non-anesthetized leg of the same animal. Therefore plain bupivacaine was not used as a reference solution. In addition, the current study design would enable bupivacaine toxicity to be avoided.

The previously described poly(sebacic co ricinoleic acid) prolonged both motor and sensory block of bupivacaine to 30 h (6). The new castor oil based formulation caused prolonged sensory anesthesia to 48 h and 24 h of motor block. Absence of sensory blockade in the animal tested at 48 h does not mean that the bupivacaine is no longer released from the polymer. *In vitro* release of 10% bupivacaine from the polymer was highest in the initial hours. Following this burst, bupivacaine release continued at a constant level, and it is possible that *in vivo* this translated to sub-therapeutic bupivacaine levels such that the sensory block was no longer apparent after 48 h despite continued bupivacaine release.

In conclusion, increased duration of the analgesia to 48 h provided by bupivacaine incorporated into a polymer carrier was possible by manipulation of the polymer properties. Future study should further investigate this drug delivery system, including stability of the formulation during storage, and reliability of manufacturing in order to assess the possibility of making such a formulation commercially available.

ACKNOWLEDGMENTS

This research was supported in part by grant no. 5868 from the Chief Scientist Office of the Ministry of Health, Israel and in part by a grant from the Joint Research Fund of the Hebrew University and Hadassah Medical Organization, Jerusalem, Israel. We are grateful to Professor Michael Tal, Department of Anatomy and Cell Biology, Hadassah Hebrew University School of Medicine, for use of his Hargreaves Hot Plate.

REFERENCES

1. G. J. Grant, B. Piskoun, and M. Bansinath. Analgesic duration and kinetics of liposomal bupivacaine after subcutaneous injection in mice. *Clin. Exp. Pharmacol. Physiol.* **30**:966–968 (2003). doi:10.1111/j.1440-1681.2003.03933.x.
2. J. Curley, J. Castillo, J. Hotz, M. Uezono, S. Hernandez, J. O. Lim, J. Tigner, M. Chasin, R. Langer, and C. Berde. Prolonged regional nerve blockade injectable biodegradable bupivacaine/polyester microspheres. *Anesthesiology.* **84**:1401–1410 (1996). doi:10.1097/00000542-199606000-00017.
3. D. R. de Araujo, C. M. Cereda, G. B. Brunetto, L. M. Pinto, M. H. Santana, and E. de Paula. Encapsulation of mepivacaine

prolongs the analgesia provided by sciatic nerve blockade in mice. *Can. J. Anaesth.* **51**:566–572 (2004).
4. D. B. Masters, C. B. Berde, S. K. Dutta, C. T. Griggs, D. Hu, W. Kupsky, and R. Langer. Prolonged regional nerve blockade by controlled-release of local anesthetic from a biodegradable polymer matrix. *Anesthesiology.* **79**:340–346 (1993). doi:10.1097/00000542-199308000-00020.
5. G. J. Grant, Y. Barenholz, B. Piskoun, M. Bansinath, H. Turndorf, and E. M. Bolotin. DRV liposomal bupivacaine: preparation, characterization, and *in vivo* evaluation in mice. *Pharm. Res.* **18**:336–343 (2001). doi:10.1023/A:1011059131348.
6. A. Shikanov, A. J. Domb, and C. F. Weiniger. Long acting local anesthetic polymer formulation to prolong the effect of analgesia. *J. Control. Rel.* **117**:97–103 (2007). doi:10.1016/j.jconrel.2006.10.014.
7. A. R. Gennaro (Ed.), 19 th ed., Remington: The Science and Practice of Pharmacy, vol. I (35),Mack Publishing Company, Easton, Pennsylvania, 1995, p. 594.
8. Proceedings of the International Conference on Harmonization (ICH), Topic Q2B: Validation of Analytical Procedures: Methodology, 1996.
9. K. Hargreaves, R. Dubner, F. Brown, C. Flores, and J. Joris. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain*. **32**:77–88 (1988). doi:10.1016/0304-3959(88)90026-7.
10. R. Slivniak, A. Ezra, and A. J. Domb. Hydrolytic degradation and drug release of ricinoleic acid-lactic acid copolyesters. *Pharm. Res.* **23**:1306–1312 (2006). doi:10.1007/s11095-006-0140-x.
11. M. Y. Krasko, and A. J. Domb. Hydrolytic degradation of ricinoleic-sebacic-ester anhydride copolymers. *Biomacromolecules*. **6**:1877–1884 (2005). doi:10.1021/bm049228v.
12. A. Shikanov, and A. J. Domb. Poly(sebacic acid-co-ricinoleic acid) biodegradable injectable *in situ* gelling polymer. *Biomacromolecules*. **7**:288–296 (2006). doi:10.1021/bm050648+.
13. M. Sokolsky-Papkov, and A. J. Domb. Stereoisomeric effect on *in vitro* drug release from injectable poly(lactic acid co castor oil) polyesters. *Polym. Adv.Technol.* **19**:1–9 (2008).