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Preparation and characterization of bupivacaine-loaded polylactide and polylactide-co-glycolide microspheres

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Abstract

Various bupivacaine-loaded microsphere systems have been prepared from polylactide-co-glycolide (PLGA) and from blends of different molecular weight polylactide (PLA) by a solvent evaporation-extraction method. In vitro drug release profiles displayed significant differences between polymers. Among PLA microspheres, the initial release was accelerated with increasing proportion of low molecular weight PLA. Preliminary pharmacokinetic studies following intrathecal and intraperitoneal administration of different bupivacaine-loaded microspheres in rabbits illustrated the controlled release of this drug.

Key words: Bupivacaine; Polylactide; Polylactide-co-glycolide; Microsphere; Controlled release; Intrathecal administration; Intraperitoneal administration

1. Introduction

Local anesthetic drugs are used for regional anesthesia and for regional control of major pain (Benumof, 1992). In this respect, they are administered either via central routes, i.e., spinal and epidural routes (Burm, 1989), or via peripheral routes (Mann et al., 1992; Narchi et al. 1992). The current increasing use of such techniques results from a decrease in the systemic administration of narcotic drugs that lead to more frequent and more severe adverse effects (Martindale, 1993; Sjöberg et al., 1992). However, regional administration of local anesthetic drugs should be improved by the development of drug delivery systems leading (i) to sustained release of the drug allowing a longer duration of action, and (ii) to slower uptake in the systemic circulation allowing an improvement of their therapeutic index (Stanley, 1988).

A number of recent works dealing with the controlled release of local anesthetic drugs highlight the interest in this problem. Several approaches have been investigated in order to control the disposition of local anesthetic drugs: influence of pH on ionization (Chestnut et al., 1989; Bonhomme et al., 1992), lipid solution (Langerman et al., 1991), suspension (Korsten et al., 1991), liquid-solid emulsion gel (Mhando and Li Wan Po, 1990), polymer carrier (Kolli et al.,

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1992), biodegradable implant (Masters et al., 1991), lipid drug carrier (Langerman et al., 1992a,b), liposomes (Gesztes et al., 1988; Legros et al., 1990; Mashimo et al., 1990, 1992) and microspheres (Wakiyama et al., 1981a,b, 1982; Kojima et al., 1984; Nakano et al., 1984; Fitzgerald et al., 1988). It is noteworthy that most of these works have described the prolongation of a pharmacodynamic effect but have not been fully characterised from a biopharmaceutical standpoint.

The objectives of this investigation were to prepare biodegradable local anesthetic drug-loaded microspheres using bupivacaine as a model drug, to show that drug release could be controlled, and to perform preliminary pharmacokinetic studies following administration of different bupivacaine-loaded microspheres via central and regional routes in animals before carrying out more complete biopharmaceutic and pharmacodynamic studies.

2. Materials and Methods

2.1. Materials

Copoly(dl-lactic/glycolic acid) (PLGA) (Resomer RG858, 85:15, Mol. Wt 48000; Resomer RG755, 75:25, Mol. Wt 15000; Resomer RG503, 50:50, Mol. Wt 9000 and Resomer RG504, 50:50, Mol. Wt 12000) and poly(dl-lactic acid) (PLA) (Resomer R104 Mol. Wt 2000 and R202 Mol. Wt 9000) were supplied by Boehringer Ingelheim (Saint Germain en Laye, France). The molecular weight was determined by the manufacturer by measurement of the intrinsic viscosity.

Bupivacaine hydrochloride was supplied by Astra (Nanterre, France). Bupivacaine was encapsulated as the base which was obtained by precipitation in alcaline medium (ammonium hydroxide) from a saturated aqueous solution of bupivacaine hydrochloride. The precipitate was rinsed with distilled water until the filtrate became neutral. Bupivacaine base was then dried under strong vacuum (0.02 atm.) at ambient temperature for 48 h. The purity of bupivacaine base (98.4%) was assessed using HPLC by comparison with bupivacaine hydrochloride.

The following chemicals were obtained from commercial suppliers and used as received: polyvinyl alcohol Rhodoviol® 4/125 and Tween 20 (Prolabo, Paris, France), dichloromethane RPE-ACS and hydrochloric acid 23% RPE (Carlo Erba, Milan, Italy), sodium carboxymethylcellulose (Cooper, Melun, france), and Tris and ammonium hydroxide 32% (Merck, Darmstadt, Germany). Polyamide membrane filters of 1 and 50 μm pore size were used (ZBF, Rüschliken, Switzerland).

2.2. Preparation of microspheres

Microspheres were prepared by using a solvent evaporation/extraction method. Solutions of bupivacaine base (500 mg) and of polymer (500 mg) in CH_2Cl_2 (5 ml) were prepared and cooled to 15°C using a Huber (Offenburg, Germany) Model Ministat cooler. The temperature was then maintained at 15°C during the phases of emulsification and elimination of the solvent. The continuous phase was a 200 ml, pH 8.0 aqueous solution containing 0.01 M Tris and 0.01% polyvinyl alcohol. The dispersed phase was poured slowly (1 min) through a nozzle of about 1.5 mm diameter into the continous phase and emulsified for 5 min using a Polytron Model PT 3000 (Kinematica AG, Luzern, Switzerland) equipped with a Model PT-DA-3030/4 dispersing tool. To eliminate dichloromethane, the emulsion was diluted progressively (1 min) with distilled water (800 ml, 15°C) and stirred for 20 min using a Model 35003 magnetic stirrer (Bioblock Scientific, Strasbourg, France). During the final 15 min, the stirring was performed under vacuum using a Model VDE-0530 pump (ABM GmBH, Marktredwitz, Germany).

After removing particles larger than 50 μm by sieving, the microspheres were collected by filtration on a 1 μm pore size membrane under a positive nitrogen pressure and rinsed twice with 1000 ml distilled water at ambient temperature. The resulting microspheres were lyophilized into a powder (Model Alpha, Chriss, Osterode/Harz, Germany) and then stored under vacuum at 4°C. For in vitro and in vivo release studies, the microspheres were suspended in an aqueous solution

containing mannitol (2.5%), sodium carboxymethylcellulose (0.75%) and Tween 20 (0.05%). Bupivacaine-loaded microspheres were prepared either with a single polymer (PLA or PLGA) or with blends of PLA (R104 and R202).

2.3. Microsphere characterisation

2.3.1. Optical microscopy

The shape and size of all batches of microspheres were evaluated under an optical microscope with transmitted light at a magnification of 1000×. Size distribution was evaluated at the micron level and was determined following observation of 200 particles.

2.3.2. Scanning electron microscopy

The particle morphology and surface characteristics were studied by scanning electron microscopy. The samples were suspended in water and one drop was placed on the aluminium sample holder, dried at room temperature and sputtered with gold/palladium using a Model JFC 1100 ion sputter (Jeol Co. Ltd, Tokyo, Japan) (1.2 kV, 7.5 mA, 5 min). A Model JSM 6400 electron microscope equipped with a camera was used (6 kV, 2000×).

2.3.3. Drug content

Bupivacaine-loaded microspheres (20 mg) were dissolved in dichloromethane (1 ml). Bupivacaine was then extracted with 0.1 N H_2SO_4 (5 ml). After shaking for 2 min and centrifugation at $900 \times g$ for 5 min, an aliquot of the aqueous phase (50 μl) was diluted in 2 ml of the mobile phase; 20 μl of the resulting solution were injected onto the chromatograph. The chromatographic conditions were as described in section 2.4.

2.4. Bupivacaine determination

HPLC analysis of bupivacaine was performed for the evaluation of the purity of the drug as a base, drug content and drug plasma concentrations. A plasma sample (1 ml), to which was added etidocaine as internal standard, was alkalinized with sodium hydroxide (100 μl, 1 M). Then, 3 ml of a heptane-ethyl acetate mixture (90:10, v/v) were added, the tube was shaken for 2 min and centrifuged at $1200 \times g$ for 10 min. A 2.5 ml aliquot of the organic phase was transferred to a conical tube containing 50 μl of 0.05 M H_2SO_4. After shaking for 1 min, the tube was centrifuged at $900 \times g$ for 5 min and the organic phase was discarded. The aquous acid phase was buffered at pH 4.0 by transfer in a tube into which a methanolic solution of sodium acetate (50 μl, 0.2 M) was previously evaporated. A 40 μl sample was injected onto the chromatographic system.

The HPLC system consisted of a Waters (Milford, MA, U.S.A.) Model 6000 pump equipped with a Waters model WISP 710 B automatic injector, an LDC Milton Roy (Riviera Beach, FL, U.S.A.) Model 3100 Spectromonitor variable wavelength detector set at 205 nm and a Delsi (Suresnes, France) Model Enica 21 integrator. Analyses were performed with a Waters Model μBondapack C18 column maintained at 30°C using as the mobile phase an acetonitrile (0.01 M)-pH 4.0 potassium dihydrogenophosphate mixture (40:60, v/v) at a flow rate of 1 ml/min. The detection limit was 5 ng/ml. The between-day reproducibility determined at a bupivacaine base concentration of 100 ng/ml was 5.6% ($n = 10$). The linearity was assessed in the concentration range 5–1000 ng/ml ($r^2 = 0.9996$).

2.5. In vitro release studies

In vitro release studies of bupivacaine were carried out with the USP XXI rotating paddle apparatus at 100 rpm and 37°C under sink conditions in 1000 ml of 0.2% NaCl aqueous solution adjusted to pH 2.0 with 25 M HCl (bupivacaine base solubility about 40 mg/ml at pH 2.0). A weighed amount of microspheres corresponding to 10 mg of bupivacaine base was suspended in test tubes with 1 ml of an aqueous solution containing mannitol (2.5%), sodium carboxymethylcellulose (0.75%) and Tween 20 (0.05%). The suspension was then poured into the release medium. Bupivacaine concentration was measured continuously at 205 nm and recorded every 5 min by using a Milton Roy (Rochester, NY,

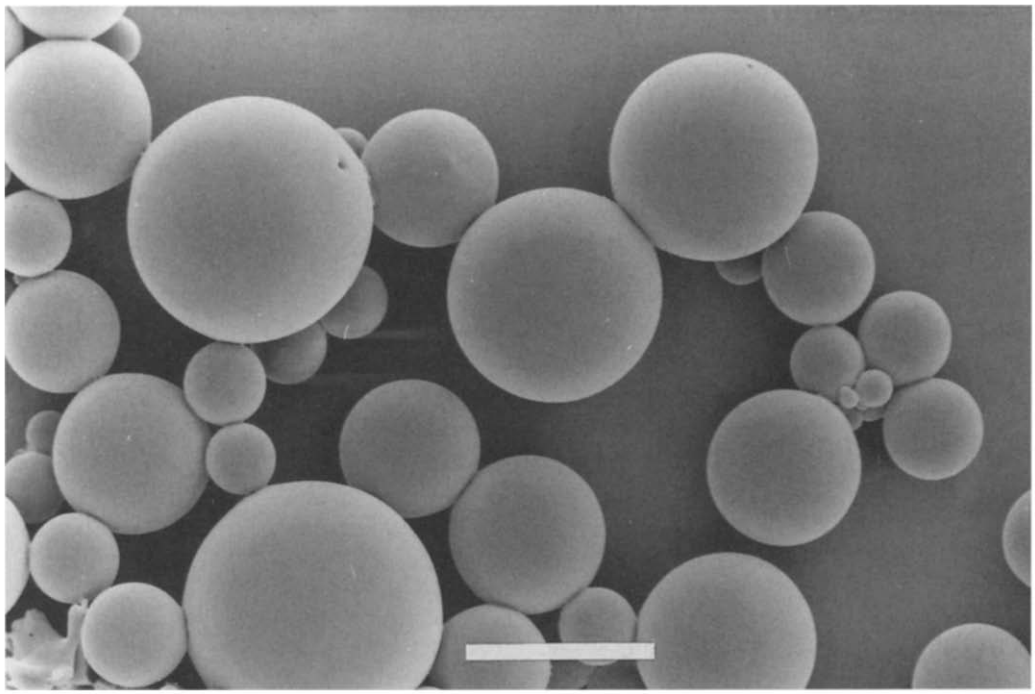

Fig. 1. Scanning electron micrograph of bupivacaine loaded microspheres prepared with PLA R104 (bar, 5 μm).

U.S.A.) Model 1201 Spectronic spectrophotometer. All samples were run at least in triplicate.

2.6. *In vivo kinetic studies*

New Zealand male rabbits (2.5–3 kg) were used. The following routes of administration were studied: intraperitoneal (i.p.) and intrathecal (i.t.). In each study, PLA R104 bupivacaine-loaded microspheres, PLGA RG755 bupivacaine-loaded microspheres and bupivacaine hydrochloride solution were administered to two animals with a 5 day wash-out period between each administration. The first administration via the intrathecal

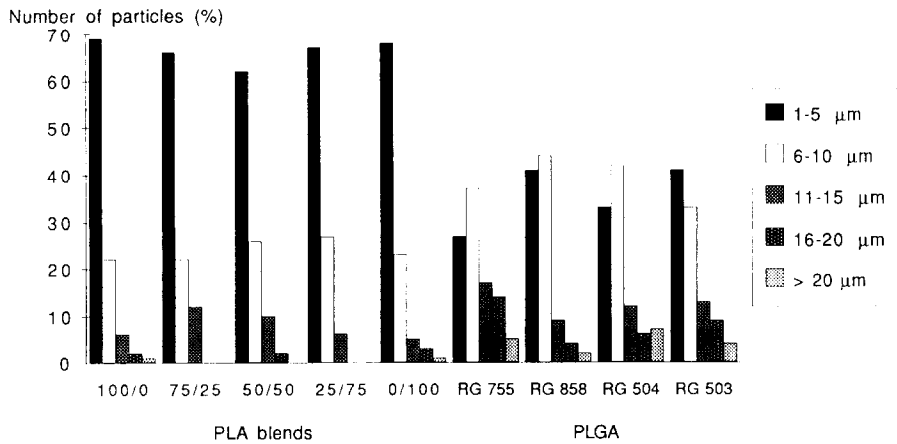

Fig. 2. Size distribution of bupivacaine-loaded polylactide and polylactide-co-glycolide microspheres.

route was achieved 48 h following insertion of a catheter according to the method of Langerman et al. (1990). The doses of bupivacaine base administered via the i.t. route as solution, PLA microspheres and PLGA microspheres were 5, 20 and 20 mg, respectively. Following the i.p. route of administration, the doses of bupivacaine base administered were 20, 80 and 80 mg, respectively.

2-ml blood samples were drawn from the marginal vein of the ear immediately before administration and then at 0.08, 0.17, 0.50, 0.75, 1, 2, 4, 6, 8 and 24 h. After centrifugation at $900 \times g$ for 5 min, plasma samples was frozen at −18°C until analysis.

3. Results and discussion

3.1. Shape and size

All batches of bupivacaine-loaded microspheres displayed smooth and spherical particles without evidence of aggregation. A scanning electron micrograph of bupivacaine-loaded microspheres prepared with PLA R104 is presented in Fig. 1. As demonstrated by Fig. 2, the size distribution was homogeneous within PLA polymers and was not affected by mixing PLA polymers. Most of the particles (about 70%) were in the range 1–5 μm. PLGA microspheres had a different size distribution. Indeed, most of the particles (about 35%) were in the ranges 1–5 and 6–10 μm.

3.2. Drug content and yield

The microsphere recovery yield was determined according to: yield (%) = 100 × mass of microspheres/(mass of drug + mass of polymer). The batch-to-batch reproducibility of the yield, evaluated using microspheres made with PLA R104, was 36.9 ± 2.2% ($n = 7$).

The homogeneity of drug content within a batch of drug-loaded microspheres was checked by measurements of bupivacaine in samples taken from a batch. The drug content (mean ± SD) of a PLA R104 batch of microspheres was 21.5 ± 0.9% ($n = 8$; lowest–highest values, 20.0–22.5%). The batch-to-batch reproducibility of drug content was evaluated by measurement of the drug content of a sample issued from different PLA R104 batches of microspheres ($n = 8$). The drug content was 21.7 ± 1.5% (lowest–highest values, 18.9–23.6%). The low variability in drug content – within batches (c.v. = 4.0%) and between batches (c.v. = 6.6%) – as well as in the recovery yield (c.v. = 6.0%) indicated the suitability of the fabrication process.

The speed of stirring during the emulsification phase using PLA R104 marginally affected the microsphere drug content. Drug contents following stirring at 2000, 3000 and 4000 rpm were 21.7, 19.9 and 19.0%, respectively. Since the microsphere size decreased with increasing speed of stirring, the decrease in drug content should result from a greater surface area allowing the drug to leach more readily during the emulsification phase.

Microspheres were made from PLA blends in PLA R104/PLA R202 mass ratios of 100:0, 75:25, 50:50, 25:75 and 0:100. In designations of mass ratios, the low molecular weight polymer is listed first. The drug contents of microspheres made from blends of PLA were quite comparable (Table 1). The difference in polymer weights of PLA should result in a differing hydrophilicities of the microspheres as a result of the difference in the hydrophilic end region (Heya et al., 1991).

Table 1
In vitro release characteristics of bupivacaine-loaded microspheres prepared with various polylactide-co-glycolide polymers (PLGA RG858, PLGA RG755, PLGA RG503, PLGA RG504) and with blends of polylactide (PLA R104, Mol. Wt 2000; PLA R202, Mol. Wt 9000) in different mass ratios

	Formulation	$T_{10\%}$ (h)	$T_{50\%}$ (h)	Drug content (%)
PLA blends (R104-R202)	100– 0	0.17	3.0	21.7
	75– 25	0.33	10.5	20.7
	50– 50	0.90	25.7	23.1
	25– 75	2.20	34.9	20.7
	0–100	5.20	ND	23.0
PLGA	RG 503	0.11	11.4	49.6
	RG 504	0.05	0.38	48.7
	RG 755	20.7	54.4	45.7
	RG 858	35.2	ND	45.3

However, this difference in microsphere hydrophilicity did not influence the drug content. A decrease in the amount of organic solvent leading to a more rapid rate of precipitation of the polymer at the surface of the droplets during the emulsification phase was evaluated in order to increase the drug content of the microspheres. However, the greater solution viscosity precluded the use of the method by which the organic phase was added to the continuous phase. High polymer solution viscosity is of interest by virtue of the increase in precipitation rate and decrease in the rate of diffusion of solvent, non-solvent and drug (Bodmeier and McGinity, 1988).

Microspheres made from various PLGA displayed a significantly higher drug content that almost reached the theoretical maximum (50%). The drug contents of microspheres made from RG 755, RG 858, RG 503 and RG 504 were 45.7, 45.3, 49.6 and 48.7%, respectively. The higher drug content achieved with PLGA polymers in comparison with PLA could be attributed to a faster rate of precipitation of the polymer at the droplet interface precluding drug partitionning into the aqueous phase. Indeed, further drug loss is not expected to occur once the polymer has precipitated at the droplet surface (Bodmeier and McGinity, 1988).

3.3. *In vitro release profiles*

The reproducibility of the in vitro release process was checked by analysis of samples ($n = 5$) of a batch of bupivacaine-loaded PLA R104 microspheres. The coefficient of variation of the cumulative percent released, determined at each measurement time, ranged between 2.6 and 6.6% indicating the suitability of this technique.

In vitro release kinetics of PLA and PLGA bupivacaine loaded microspheres displayed significant differences between polymers (Table 1). In particular, the kinetics of bupivacaine release from PLA R104 microspheres displayed two phases, suggesting different mechanisms of drug

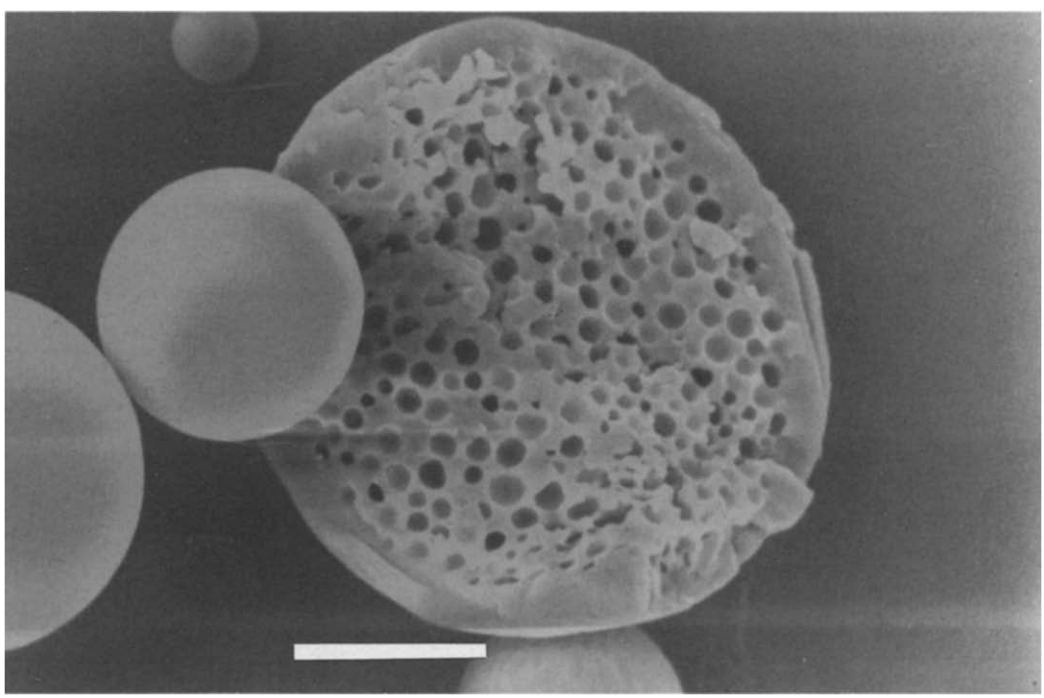

Fig. 3. Scanning electron micrograph of the inner structure of a bupivacaine-loaded microsphere prepared with PLA-R104 (bar, 5 μm).

release. The initial stage led to rapid release ('drug-burst) with about 51.3 ± 3.4% (mean ± SD, $n = 5$) of drug released during the first 3 h. This phase was followed by an apparent linear phase, indicative of zero-order kinetics, during which drug release was rather low, about 1.1 ± 0.1% per h. The initial release is unlikely to result from dissolution of crystalline bupivacaine on the surface of the microspheres. Indeed, crystals of bupivacaine were not observed using SEM (under particular experimental conditions crystallisation of bupivacaine can be observed). Moreover, dissolution of free bupivacaine base is very rapid (93.5% in 15 min) under these in vitro release conditions. Owing to its high rate, the initial release should not be controlled by drug diffusion within the polymer matrix or polymer degradation. It may result from dissolution of the drug within fluid-filled pores located near the surface of the microspheres. Indeed, SEM observation of a batch of PLA R104 microspheres demonstrated a broken microsphere displaying a porous inner structure (Fig. 3). As drug dissolves it leaves channels for rapid diffusion of fluid into, and drug out of, the inner regions of the microspheres. In this initial phase, during which drug release may occur according to a pore diffusion mechanism through an interconnecting network, the rate-limiting step may be the penetration of fluid into the microspheres. On the other hand, the drug release during the apparent linear phase may result from polymer degradation, from drug diffusion through the polymer matrix or both.

Blending of the two PLA polymers allowed the regulation of drug release (Table 1). The drug content and size distribution were very similar between PLA blends and can be ruled out as the cause of the differences in release rates. The increase in the proportion of low molecular weight PLA (MW-PLA) resulted in an increase in the release rate of the drug (Table 1). The PLA polymer glass transition temperature is influenced by the molecular weight, especially in the case of the lowest molecular weights (Omelczuk and McGinity, 1992). The influence of the glass transition temperature on the physical and mechanical properties of polymers may explain the difference in release rates observed between PLA blends, the lower glass transition temperature leading to the faster release rate (Bodmeier et al., 1989). However, other factors should be considered to explain bupivacaine release from PLA blends, e.g., the solubility of the drug in the polymer matrix, drug-polymer charge interaction between ionized carboxyl groups of PLA and the ionized tertiary amine group of bupivacaine and possibly leaching of low molecular weight PLA during the release study.

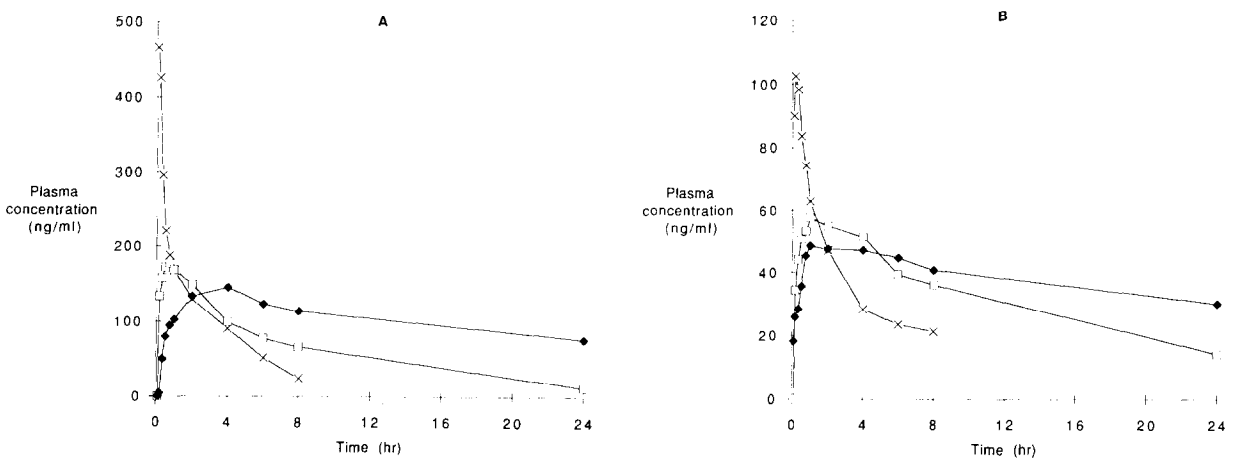

Fig. 4. Bupivacaine plasma concentrations following administration of bupivacaine via intrathecal (A) and intraperitoneal (B) routes in rabbits: free drug (×), R104 PLA bupivacaine-loaded microspheres (□) and RG755 PLGA bupivacaine-loaded microspheres (◆).

A detailed investigation of the inner structure of the microspheres as well as differential scanning calorimetric analysis are needed to characterize the status of the drug inside the microspheres and to gain further insights into the mechanism of drug release.

3.4. *In vivo kinetic profiles*

Plasma concentration vs time curves following intrathecal and intraperitoneal administration of bupivacaine solution and bupivacaine-loaded microspheres (PLA R104 and PLGA RG755) are illustrated in Fig. 4. The maximum plasma concentrations (C_{max}) following administration of bupivacaine solution, PLA R104 and PLGA RG 755 microspheres via the intrathecal route were 470, 177 and 146 ng/ml, respectively. Following intraperitoneal administration, C_{max} values were 103, 58 and 48 ng/ml, respectively. These reductions in C_{max} should result from slower uptake of bupivacaine in the systemic circulation. Furthermore, the shape of the apparent elimination phase of the plasma concentration vs time curves suggests sustained release of the drug. A complete biopharmaceutic and pharmacodynamic study of bupivacaine-loaded microspheres will soon be reported.

In conclusion, bupivacaine-loaded microspheres were successfully prepared from various polylactide-co-glycolide polymers and from blends of polylactide with different molecular weights allowing control of the release rate. This preliminary in vivo study highlights the interest in such a drug delivery system for circumventing the problems inherent in the use of local anesthetic drugs for regional anesthesia and for regional control of pain.

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