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Spray-dryed bupivacaine-loaded microspheres: in vitro evaluation and biopharmaceutics of bupivacaine following brachial plexus administration in sheep

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Abstract

Microspheres could be used as a drug delivery system to prolong the duration of action of bupivacaine and to reduce its systemic absorption leading to high plasma concentrations related to central nervous and cardiovascular toxicity. Bupivacaine-loaded microspheres were made by spray-drying using polylactide-co-glycolide polymers from different sources and with different bupivacaine–polymer ratio. The characterization of microspheres concerned the shape and size, the bupivacaine drug-content (DC) and the cumulative release profiles. We evaluated in sheep the bupivacaine pharmacokinetics: (i) after short intravenous infusion of 75 mg bupivacaine solution; and (ii) following brachial nerve plexus injections of 75 mg bupivacaine solution alone, with the addition of 75 μg epinephrine, with the addition of 150 μg epinephrine and of bupivacaine (750 mg)-loaded microspheres. Release profiles showed a biphasic pattern whatever the DC. After i.v. infusion the mean clearance value was 1.53 ± 0.53 l/min and the mean elimination half-life was 120.5 ± 73.1 min. Following brachial plexus nerve injection, bupivacaine C_{max} were lower than 100 ng/ml following either solution or microspheres administration. Ninety percent of the 75 mg bupivacaine given as a solution were absorbed in 5.8 ± 1.0 h (bupivacaine alone) compared to 24.6 ± 1.2 h following microsphere administration.
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Keywords: Spray-dried microspheres; Bupivacaine; Controlled release; Plexus administration; Sheep

1. Introduction

Local anesthetics are often used for regional control of major postoperative pain. However, their use is limited by their relatively short duration of action requiring repeated administrations through indwelling catheters, and by their sys-

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temic toxicity related to high drug plasma concentrations resulting from a fast systemic absorption (Naguib et al., 1998). Improvement of regional administration of local anesthetics could be obtained by their incorporation in controlled delivery systems such as implants (Masters et al., 1993; Blanco et al., 1999), liposomes (Boogaerts et al., 1995; Malinovsky et al., 1999), drug complexes with cyclodextrins (Dollo et al., 1998) or microparticles (Le Corre et al., 1994; Curley et al., 1996; Kohane et al., 2000). Among these drug delivery systems, microspheres are interesting because of their ability to provide a controlled release rate of the drug and their small particular size allowing local injection through needles. Such bupivacaine microspheres could lead to a sustained release of the drug allowing a longer duration of action and to a slower uptake in the systemic circulation avoiding high plasma concentrations. Recent in vivo studies dealing with the controlled delivery of local anesthetic in analgesia highlight the interest for this problem (Curley et al., 1996; Castillo et al., 1996; Le Corre et al., 1997; Dräger et al., 1998; Garry et al., 1999; Malinovsky et al., 1999). Bupivacaine-loaded microspheres have been prepared by a spray-drying process presenting some advantages, i.e. one-step, fast and suitable for scale-up, compared to usual microencapsulation methods (emulsification, phase-separation, and coacervation methods) (Conte et al., 1994). Moreover, the spray-drying method provides high entrapment efficiencies, narrow size distributions with small particle sizes and low levels of residual organic solvent (Benoit, 1996; Bitz and Doelker, 1996). Improved pharmacodynamics of bupivacaine after bupivacaine-loaded microspheres administration has been previously reported after spinal administration in rabbits (Le Corre et al., 1995; Malinovsky et al., 1997), after subcutaneous injection in an acute inflammatory pain model in rats (Fletcher et al., 1997) and after plexus brachial administration in sheep (Estébe et al., 2001). The purpose of our study was: (i) the preparation and characterization of bupivacaine-loaded microspheres prepared by a spray-drying process; (ii) the evaluation of the biopharmaceutics of bupivacaine after peripheral administration near the brachial plexus nerve in sheep, which is comparable in body weight and length of nerves to adult humans.

2. Materials and methods

2.1. Materials

D,L-Polylactide-co-glycolide polymers (PLGA): Resomer RG503H (50:50, M.W. 9 kDa), Resomer RG 503 (50:50, M.W. 9 kDa), Resomer RG 504 (50:50, M.W. 12 kDa), Resomer RG 755 (75:25, M.W. 15 kDa) were supplied from Boehringer Ingelheim (Saint Germain-en-Laye, France) and PLGA polymers (50:50 low i.v., M.W. 63 kDa and 65:35 high i.v., M.W. 141 kDa) were supplied from Alkermes (Ohio, USA). Bupivacaine hydrochloride was supplied by Astra (Nanterre, France). Bupivacaine was encapsulated as the base obtained by precipitation in an alkaline medium (ammonium hydroxide) from a saturated aqueous solution of bupivacaine hydrochloride form. The purity of the resulting bupivacaine base was checked using HPLC by comparison with bupivacaine hydrochloride. Epinephrine was supplied by Aguettant (Lyon, France).

The following chemicals were used as received: methylene chloride RPE-ACS, 85% phosphoric acid, 23% hydrochloric acid, mannitol, Heptane RPE, potassium monobasic phosphate, potassium hydrogenophosphate (Carlo Herba, Milan, Italy); sulfuric acid, sodium hydrochloride (Merck, Darmstadt, Germany); acetonitrile HPLC-S (Biosolve, Valkenswaard, Netherlands); Tween 20 (Prolabo, Paris, France); sodium carboxymethylcellulose (Cooper, Melun, France).

2.2. Preparation of microspheres

Bupivacaine-loaded microspheres were prepared by dissolving bupivacaine base and polymer in methylene chloride 4% (w/w) for polymer from Boehringer Ingelheim and 2% (w/w) for polymer from Alkermes. Different bupivacaine–polymer weight ratios were used: 20–80, 30–70, 40–60, 50–50, 60–40 (wt.%) for polymer from Boehringer Ingelheim and 40–60, 50–50 (wt.%) for polymer from Alkermes. The solution was

spray-dried with a Mini Büchi B-191 laboratory spray-dryer (Büchi Laboratorium AG, Switzerland) using a 0.7 mm nozzle. The process parameters were set as follows: inlet temperature (50 °C); outlet temperature (41–43 °C); aspirator setting (100%); pump setting (2.0–2.5 ml/min) and spray flow (600 nl/h). Microspheres were kept under vacuum at 4 °C until characterization.

2.3. Characterization of microspheres

2.3.1. Size and shape

Microspheres were dispersed in 5 ml of a 0.05% Tween 20 aqueous solution, ultrasonicated for 10 s, and then dispersed in 75 ml of distilled water. After dispersion, size distribution was assessed by laser light scattering using a Malvern Mastersizer S (Malvern Instruments, Orsay, France). The size distribution parameters were the volume diameter $D(v; 0.5)$ for 50% of the sample, the average volume diameter, $D(4, 3)$ and the Span: $[D(v; 0.9) - D(v; 0.1)]/D(v; 0.5)$. Each batch was measured in triplicate. Microspheres morphology and surface characteristics were observed by scanning electron microscopy (SEM) using a Jeol JSM Model 6400 electron micrograph. The spray-dried microspheres were dispersed in a Tween 20 (0.05% in water) solution, dried at room temperature on double-faced adhesive on the metal stub and then coated with a thin layer of gold/palladium (7.5 mA, 5 min) using a Jeol JFC ion-sputter Model 1100 (Jeol, Tokyo, Japan).

2.3.2. Drug-content

Weighted samples of bupivacaine-loaded microspheres (around 20 mg) were dissolved in methylene chloride (1 ml). The drug was then extracted in 0.1 N sulfuric acid (5 ml) loaded with etidocaine as internal standard. After shaking (5 min) and centrifugation (3000 rpm, 10 min), 20 μl of the aqueous phase were diluted in 2 ml of the mobile phase and 20 μl of this diluted solution were injected into the chromatograph. HPLC analysis conditions were as previously described (Le Guevello et al., 1993) The HPLC system was composed of a Waters Model 6000 pump (Milford, MA) equipped with a Waters Wisp Model 717 automatic injector, and a LDC Milton Roy Spectromonitor Model 3100 (Riviera Beach, FL), wavelength detector set at 205 nm, and a Delsi Model Enica 21 Integrator (Suresnes, France). Analysis were achieved by using a 125 × 3 Merck Lichrospher RP-B column maintained at 30 °C. The mobile phase was a 22:78 (v/v) mixture of acetonitrile and of 0.01 M KH_2PO_4 aqueous solution acidified with 0.1% H_3PO_4, at a 0.5 ml/min flow rate.

2.4. In vitro release study

Release studies were performed by using a Distek dissolution test system Model 5100A (North Brunswick, NJ), using a rotating paddle apparatus (100 rpm). The release medium was a 0.2% NaCl aqueous solution (900 ml), adjusted to pH 2.0 with HCl and thermostated at 37 °C. Weighted amount of bupivacaine-loaded microspheres (around 20 mg) was suspended in 1 ml of an aqueous solution containing 2.5% mannitol, 0.75% sodium carboxymethylcellulose and 0.05% Tween 20 and dropped in the release medium. Percent cumulative release of bupivacaine was measured continuously at 205 nm by using a Uvikon spectrophotometer Kontron Model 922 (St Quentin Yvelines, France). Each batch of microspheres was analyzed in triplicate and data were processed using the Icalis Data System IDIS EE software (Berkshire, UK). In vitro release data (time corresponding to 10, 50 and 90% of bupivacaine release) were derived from raw data. Mean dissolution time (T_d) was derived from the fit of the percent released-time plots using the Weibull equation with the software package Simed SIPHAR (Creteil, France).

2.5. In vivo evaluation

2.5.1. Formulation of microspheres

Microspheres were suspended in 30 ml of an aqueous solution containing 5% mannitol and 0.05% Tween 20 using ultrasonication (2 min), then microspheres were rapidly frozen in ethanol (−40 °C). Microspheres were then freeze-dried for 48 h and kept at 4 °C until administration. Microspheres were suspended in 30 ml of sterile water immediately before injection.

2.5.2. Study design

The study was performed according to a protocol approved by the Local Animal Research Committee. The animals were 13 non-pregnant Lacaunes ewes, with a median age of 2.5 ± 1 years and a median weight of 73 ± 10 kg. In a first step, all sheep received a 15-min intravenous infusion of 75 mg of bupivacaine hydrochloride in 0.9% NaCl solution. In a second step, a short general i.v. anesthesia (5–8 mg/kg of thiopental) was performed in the right jugular vein. At the same time, on the left side, bupivacaine (solution or drug-loaded microspheres) was injected near the brachial nerve plexus using a nerve stimulator for precise identification of the nerve plexus location (Estébe et al., 2000). PLGA RG503H bupivacaine-loaded microspheres were chosen for the in vivo experiment because of the higher hydrophilicity of this polymer that should result in a lower phagocytosis rate at the site of injection (Torchè et al., 2000).

This second step was divided in three parts separated by a 2–3-week interval. First, ten sheep received 30 ml of a 0.9% NaCl solution of 75 mg bupivacaine hydrochloride. Second, eight sheep received 30 ml of a 75 mg bupivacaine hydrochloride in 0.9% NaCl solution supplemented with epinephrine 75 μg ($n = 4$) or 150 μg ($n = 4$). Finally, 11 sheep received 30 ml of bupivacaine-loaded microspheres containing an equivalent of 750 mg of bupivacaine hydrochloride. The bupivacaine–polymer ratio of the microspheres injected in sheep was 50–50 ($n = 8$) and 40–60 ($n = 3$).

Blood samples (7 ml) were taken from the catheter remaining in the jugular vein, of the opposite side of bupivacaine injection, just before injection and at 1, 3, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300 min after, and later (until 30 h) in sheep receiving bupivacaine-loaded microspheres. After centrifugation for 10 min at 4000 rpm, plasma samples were kept frozen (−18 °C) until analysis.

2.5.3. Plasma determination of bupivacaine

Plasma sample (0.5 ml) was loaded with etidocaine as internal standard and alkalinized with 1 N sodium hydroxide (0.05 ml). Then, heptane (2 ml) was added and the resulting mixture was shaken 5 min and centrifuged 5 min at 5000 rpm. Fifty μl of 0.1 N sulfuric acid were added in the recovered organic phase. After shaking 3 min and centrifugation 5 min at 4000 rpm, the recovered aqueous phase (0.05 ml) was buffered with 0.01 ml of dibasic potassium phosphate and 0.05 ml of this dilution was injected into the HPLC system. The chromatographic system and the HPLC conditions were as previously described (Le Guevello et al., 1993).

2.5.4. Biopharmaceutic evaluation

Following IV infusion, the central compartment distribution volume (V_c), steady state distribution volume (V_{ss}), clearance (C_L), apparent elimination half-life ($t_{1/2\beta}$), and distribution half-life ($t_{1/2\alpha}$), distribution (K_{12}, K_{21}) and elimination (K_{10}) rate constant from each individual plasma concentration data sets were obtained from a compartmental analysis assuming a first-order elimination from the central compartment with the software package WinNonlin 1.5 version (S.C.I., Apex, NC).

Following brachial administration, the peak plasma concentration (C_{max}) and corresponding time to peak concentration (T_{max}) were derived from raw data. The bupivacaine terminal apparent elimination half-life ($t_{1/2\beta}$) after brachial injection was estimated following non-compartmental analysis with the software package WinNonlin (S.C.I., Apex, NC). Individual in vivo absorption-times ($T_{10\%}$, $T_{50\%}$, $T_{90\%}$) after brachial plexus bupivacaine injection were estimated from the Loo–Riegelmann absorption analysis (Loo and Riegelman, 1968) using the software Simed SIPHAR (Creteil, France). The values of the distribution rate constants (K_{12} and K_{21}) and elimination rate constant (K_{10}) required for the Loo–Riegelman analysis, were derived from those obtained following the i.v. infusion. A mathematical derivation allowed us to determine the rate constant identical to an i.v. bolus injection curve by utilizing the postinfusion curve analysis (Loo and Riegelman, 1970). Indeed, due to potential toxicity, bupivacaine was not injected as an i.v. bolus explaining the choice to use an i.v. infusion.

3. Results and discussion

3.1. Preparation of PLGA microspheres

Spray-drying conditions were defined using bupivacaine-free microspheres batches of six PLGA polymers with different molecular weight and lactide/glycolide ratio (Boehringer Ingelheim: RG503, RG503H, RG504, RG755 and Alkermes: 50:50 low i.v. and 65:35 high i.v.). Peristaltic pump speed ranging 5–20% was used and variations in the average volume diameter $D(4, 3)$ were checked. Diameter decreased when pump speed increased and remained constant above 10%. Thus, the immediately upper value of 15% was chosen, corresponding to a feed rate of 2.0–2.5 ml/min which has been previously used in spray-drying studies (Conte et al., 1994; Gander et al., 1995). The parameters used to compare batches were the shape and the size of the microspheres. The maximum polymer concentration leading to spherical and individualized particles was 4% for

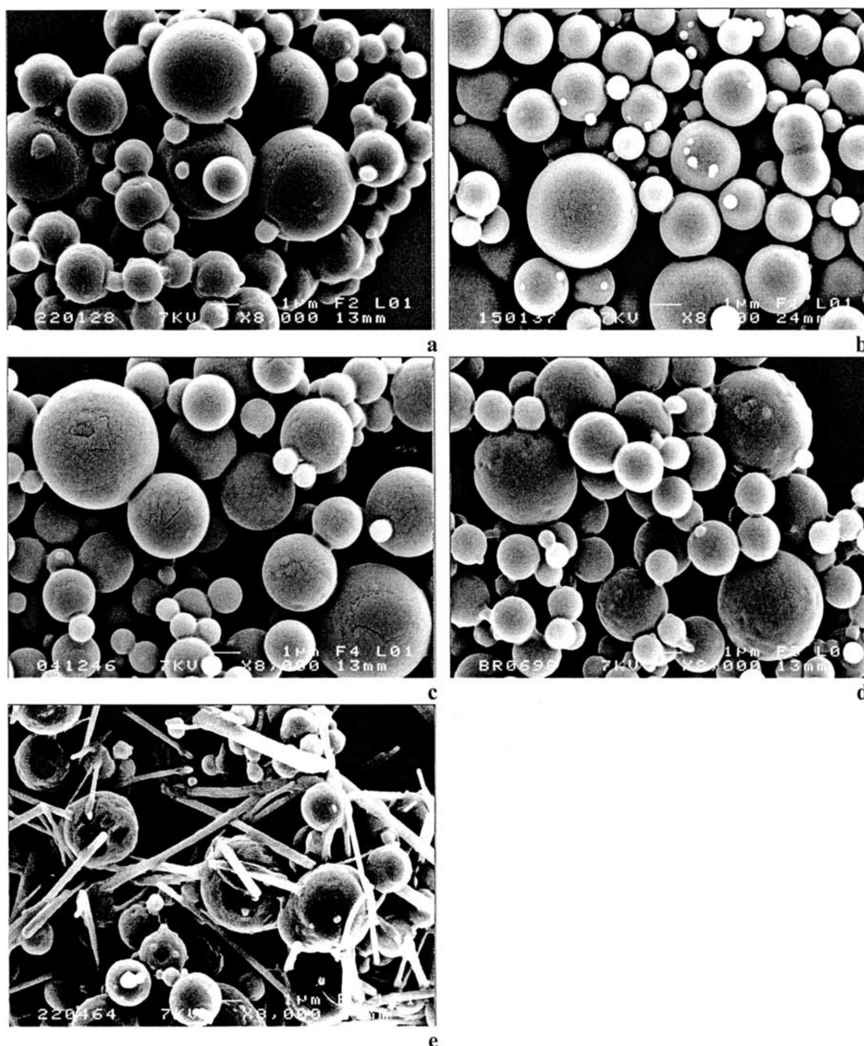

Fig. 1. Scanning-electron microscopy of bupivacaine-loaded microspheres prepared with PLGA RG503H polymer with different drug-polymer ratio (%): 50:50 (a) 20–80; (b) 30–70; (c) 40–60; (d) 50–50; (e) 60–40.

Table 1
Release kinetic parameters ($T_{10\%}$, $T_{50\%}$, $T_{90\%}$, T_d) of bupivacaine from PLGA bupivacaine-loaded microspheres and size parameters ($D(v; 0.5)$; $D(4.3)$ and Span)

Polymer	Drug-content		$T_{10\%}$ (h)	$T_{50\%}$ (h)	$T_{90\%}$ (h)	T_d (h)	$D(v; 0.5)$ (μm)	$D(4.3)$ (μm)	Span
	Theoretical (%)	Experimental (%)							
Boehringer	20	19.9	0.33	–	–	25.7	4.7	6.1	2.4
Ingelheim	30	29.9	0.33	–	–	26.7	5.6	6.9	2.5
PLGA	40-A	39.8	0.25	8.50	–	17.5	4.9	6.2	2.6
RG503H	40-B	39.6	0.25	8.50	–	17.5	5.8	7.1	2.5
	40-C	40.0	0.25	7.00	–	14.7	4.1	5.1	2.5
	50-A	51.7	0.11	1.33	–	3.3	3.3	4.5	2.6
	50-B	49.7	0.04	1.00	–	2.4	2.8	3.6	2.4
	60	59.7	0.02	0.05	0.5	0.1	2.3	3.0	2.0
Alkermes	40		0.80	>24	–	201	8.9	12.7	2.3
PLGA50:50	50-A		0.02	0.40	–	2.4	8.9	10.1	2.6
Low i.v.	50-B		0.02	0.90	–	7.7	7.3	8.4	2.2
	50-C		0.02	0.30	–	1.0	9.5	12.1	2.7
Alkermes	40		0.15	>24	–	124	4.4	5.6	2.4
PLGA65:35	50-A		0.05	12.8	–	47.8	4.4	5.0	2.1
High i.v.	50-B		0.03	7.8	–	42.9	5.1	5.9	2.2

RG503H and RG503 polymers, 3% for RG755 polymer and 2% for Alkermes polymers. Such difference amongst polymers could be related to the solution viscosity depending from the molecular weight of the polymer and/or to the glass transition temperature.

3.2. *In vitro characterization of bupivacaine-loaded microspheres*

3.2.1. *Shape and size*

Photographs of bupivacaine-loaded PLGA RG503H microspheres are presented in Fig. 1a–e. Fig. 1a–d (20–80 to 50–50) showed smooth, and regular spherical particles without aggregation. There was no particular difference between microsphere formulations prepared with different drug-content. Conversely, the Fig. 1e (60–40 bupivacaine–polymer ratio) showed incompletely formed microspheres, and some residues looking as fibers. Such residues were considered as drug and not polymer residues, since they disappeared from the SEM examination after an acidic washing of microspheres (suspension in 0.1 N HCl). This should result from too small amount of polymer to entrap bupivacaine using a 60–40 bupivacaine–polymer ratio.

Mean size of PLGA RG 503H microspheres (Table 1) tended to be smaller for those obtained with the highest polymer content (50 and 60%). While Alkermes (65:35 high i.v.) PLGA microspheres had a size similar to those obtained with PLGA RG 503H, Alkermes (50:50 low i.v.) PLGA microspheres had a higher diameter. Whatever the polymer used, the uniformity of microspheres size distribution was very similar for all batches.

3.2.2. *Drug-content*

The experimental drug-content values (Table 1) were very close to the theoretical drug-content values indicating the high encapsulation efficiency obtained using the spray drying method in contrast to lower encapsulation efficiencies obtained using the solvent evaporation/extraction method with some polymers (Le Corre et al., 1994).

3.2.3. *In vitro release profiles*

In vitro release kinetic profiles of bupivacaine-loaded microspheres showed significant differ-

ences related to the polymer and to the drug–polymer ratio (Table 1 and Fig. 2). All formulations displayed a biphasic pattern with a burst effect followed by a slow release phase as described in previous works (Le Corre et al., 1995, 1997). However, the biphasic pattern was different between the polymers as a function of their source. PLGA polymers from Alkermes Company had a more pronounced burst compared to Boehringer Ingelheim PLGA polymers (especially for a 50:50 drug content) and the release rate from the second phase appeared smaller. The main difference between these polymers resides in the burst kinetics. Indeed, it is apparent that Alkermes PLGA 50/50 microspheres released quite instantaneously part of the drug while such release rate was lower for Boehringer Ingelheim PLGA 50/50 microspheres. Differences in the amount of drug residues at the particle surface may account for such difference in the release profiles.

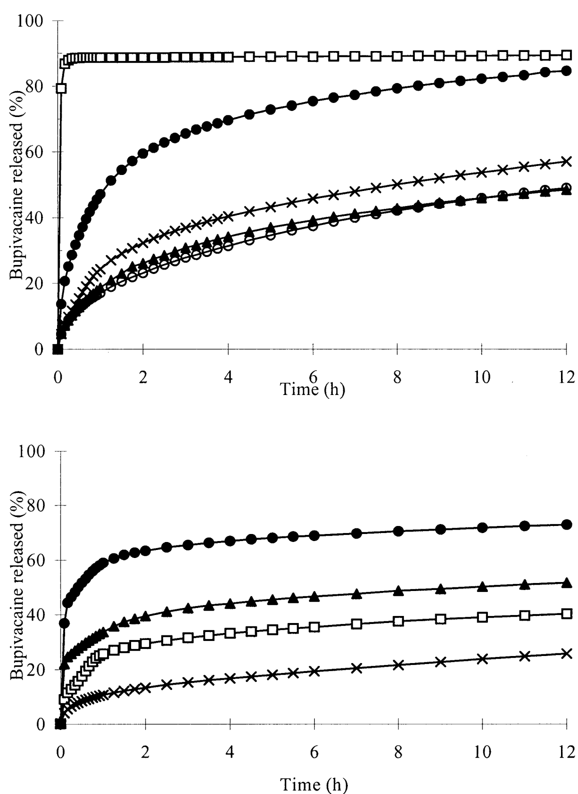

Fig. 2. Top panel: Bupivacaine cumulative release (%) from Boehringer Ingelheim PLGA RG503H microspheres (top) with different drug-polymer ratios: 20–80: ○, 30–70: ▲, 40–60: ×, 50–50: ●, 60–40: □. Bottom panel: Bupivacaine cumulative release (%) from PLGA Alkermes 50:50 low i.v. microspheres: 40–60: ×, and 50–50: ●, and from PLGA Alkermes 65:35 high i.v. microspheres: 40–60: □, and 50–50: ▲.

Experiments with PLGA RG 503H showed that the T_d decreased as a function of the polymer content highlighting the possibility to control the release rate of bupivacaine from microspheres that could be optimized by mixing different formulations. Microspheres with a bupivacaine–polymer ratio of 60–40 showed an immediate and total release of bupivacaine from microspheres (almost 90% of the drug being released in 0.05 h) in accordance with the unsuitable entrapment of bupivacaine discussed above.

3.3. Biopharmaceutic evaluation

3.3.1. Bupivacaine intravenous infusion in sheep

Plasma concentration time-course following IV infusion showed a rather low scattering (Fig. 3a). The time course displayed a biphasic pattern characterized by rapid distribution and elimination phases with an apparent distribution half-life ($T_{1/2\alpha}$) of 9.6 min and an apparent elimination half-fife ($T_{1/2\beta}$) of 121 min. The pharmacokinetic parameters (Table 2) were in very close agreement with data previously reported (Santos et al., 1997).

3.3.2. Bupivacaine brachial nerve plexus administration in sheep

Maximum bupivacaine plasma concentrations (C_{max}) obtained after brachial nerve injection either with 75 mg bupivacaine HCl solution (3 groups) or with 750 mg bupivacaine-loaded microspheres were around 100 ng/ml (Table 3, Fig. 3b–d). Time to reach C_{max} (T_{max}) were similar (around 1 h) in each group receiving bupivacaine solution (with and without epinephrine) and was much lower than those obtained after bupivacaine-loaded microspheres administration (around 6 h). Such data indicated the in vivo controlled release of the bupivacaine from the microspheres.

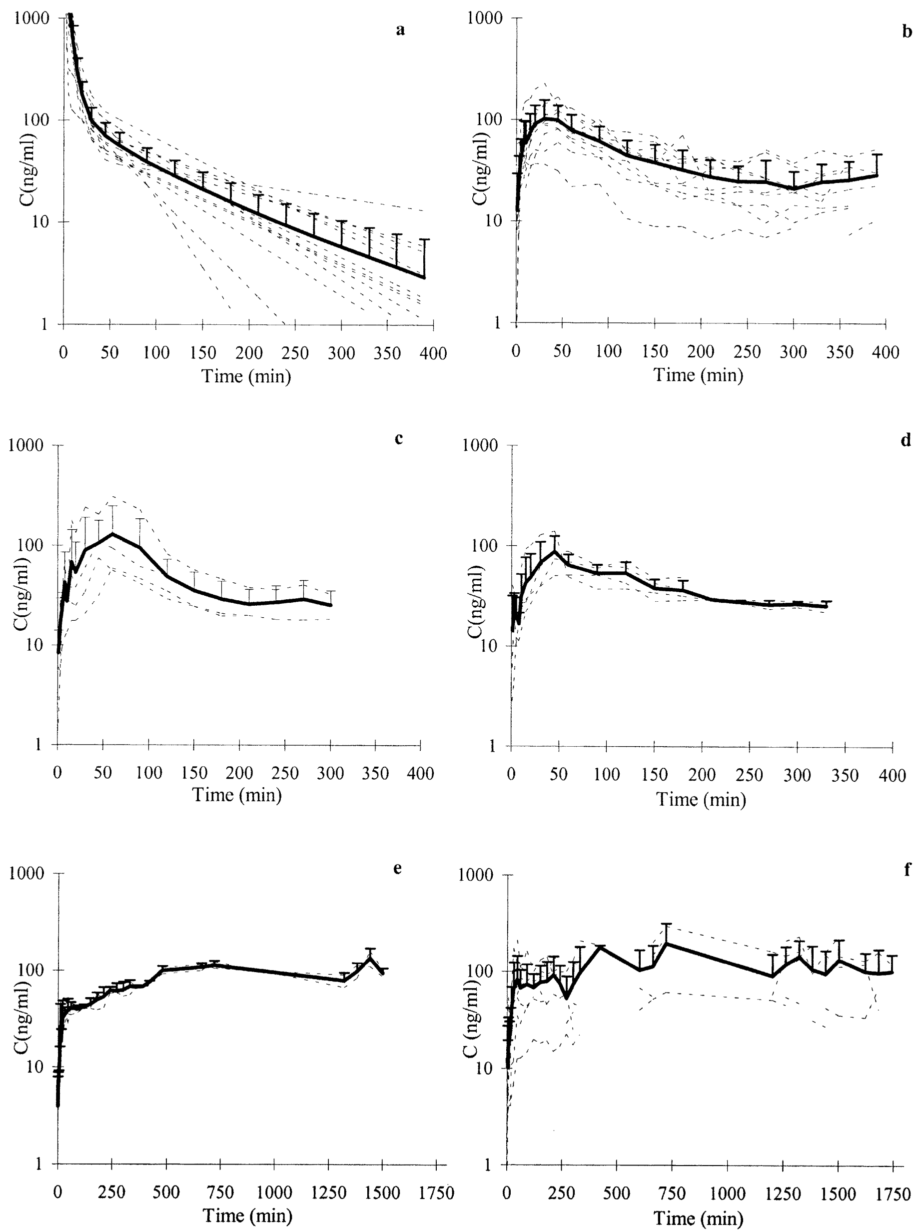

Fig. 3. Individual (dotted lines) and mean ± S.D. (full line) plasma bupivacaine concentrations (ng/ml) following: (a) 15 min i.v. infusion of 75 mg bupivacaine HCl solution ($n = 13$); (b–f) brachial plexus nerve injection in sheep of: (b) 75 mg bupivacaine HCl solution ($n = 10$); (c) 75 mg bupivacaine HCl solution with 75 μg epinephrine ($n = 4$); (d) 75 mg bupivacaine HCl solution with 150 μg epinephrine ($n = 4$); (e) 750 mg bupivacaine-loaded PLGA RG503H microspheres with a drug–polymer ratio of 40–60 ($n = 3$); (f) 750 mg bupivacaine-loaded PLGA RG503H microspheres with a drug–polymer ratio of 50–50 ($n = 8$). Bupivacaine doses are expressed in bupivacaine HCl equivalent.

The C_{max} obtained with the 40–60 bupivacaine–polymer formulation (71 ± 6 mg/l) was lower that obtained with the 50–50 bupivacaine–polymer formulation (125 ± 54 mg/l) in accordance with the in vitro differences in the release rates which are noticeable in the first hours of release.

The percent absorbed-time plots (Fig. 4a–c), as well as C_{max} and T_{max} (Table 3), of bupivacaine after administration of the solution with or without epinephrine (75 or 150 μg) were similar indicating that in our model epinephrine did not influence the absorption rate of bupivacaine. Epinephrine is usually added to local anesthetics to prolong duration of action and it has been recently shown in humans that epinephrine decreased the elimination of lidocaine from the injection site following administration close to peroneal nerves of the foot (Bernards and Kopacz, 1999).

Following brachial administration of bupivacaine as a solution, the apparent elimination half-life (around 5 h) was much higher than that observed following i.v. infusion (around 2 h) suggesting that the absorption at the brachial site from the perivascular sheath could be a rate-limiting factor in the absorption of bupivacaine. Such a flip-flop phenomenon has been shown for ropivacaine and bupivacaine in humans following brachial plexus administration (Vainionpää et al., 1995).

After bupivacaine-loaded microspheres administration, the plasma profiles displayed an apparent plateau lasting around 25 h suggesting a constant input rate of bupivacaine for both microspheres formulations (Fig. 3e–f). Such zero-order absorption is illustrated by the percent absorbed-time plots (Fig. 4d–e). Although there were differences in in vitro release rates between the two formulations of microspheres, such differences disappeared in vivo as shown by the percent absorbed-time plots. Such discrepancy can be explained by the fact that the in vitro differences were observed only in the first hours of release. Thus, in vivo differences can only be evidenced in the first hours following administration. The lower C_{max} observed with the slowest formulation can be an illustration of this phenomenon.

The prolonged zero-order absorption of bupivacaine resulting from the release of the drug from the microspheres is of paramount interest in order to obtain a constant and prolonged pharmacological effect at the site of action. In a recent report (Dräger et al., 1998) in which PLGA microspheres were injected in the intercostal area in sheep, the plasma profiles were different than those observed in the current study showing an

Table 2
Pharmacokinetic parameters following i.v. infusion (15 min) of 75 mg bupivacaine HCl solution in sheep ($n = 13$)

Sheep	V_c (l)	V_{ss} (l)	C_L (l/min)	K_{12} (min^{-1})	K_{21} (min^{-1})	K_{10} (min^{-1})	$t_{1/2\beta}$ (min)	$t_{1/2\alpha}$ (min)
S1	21	106	1.06	0.046	0.011	0.051	125	6.7
S2	24	77	1.02	0.035	0.016	0.042	87	8.1
S3	65	134	1.71	0.013	0.013	0.026	94	15.6
S4	15	98	1.68	0.093	0.017	0.112	79	3.3
S5	44	169	1.33	0.026	0.009	0.030	151	11.4
S6	4	39	0.96	0.176	0.019	0.255	63	1.6
S7	78	212	1.69	0.014	0.008	0.022	158	17.9
S8	80	227	2.40	0.035	0.019	0.030	92	9.0
S9	25	200	2.66	0.194	0.028	0.107	75	2.2
S10	26	81	0.91	0.038	0.018	0.035	92	8.4
S11	109	481	1.66	0.014	0.004	0.015	345	22.0
S12	43	157	1.58	0.046	0.018	0.036	101	7.5
S13	38	101	1.30	0.019	0.011	0.035	114	11.9
Mean	44	160	1.53	0.058	0.015	0.061	121	9.6
±S.D.	31	112	0.53	0.060	0.006	0.066	73	6.1
Santos[a]	55	190	1.66	0.048	0.019	0.030	118	8.6

[a] Data previously reported by Santos in sheep [24].

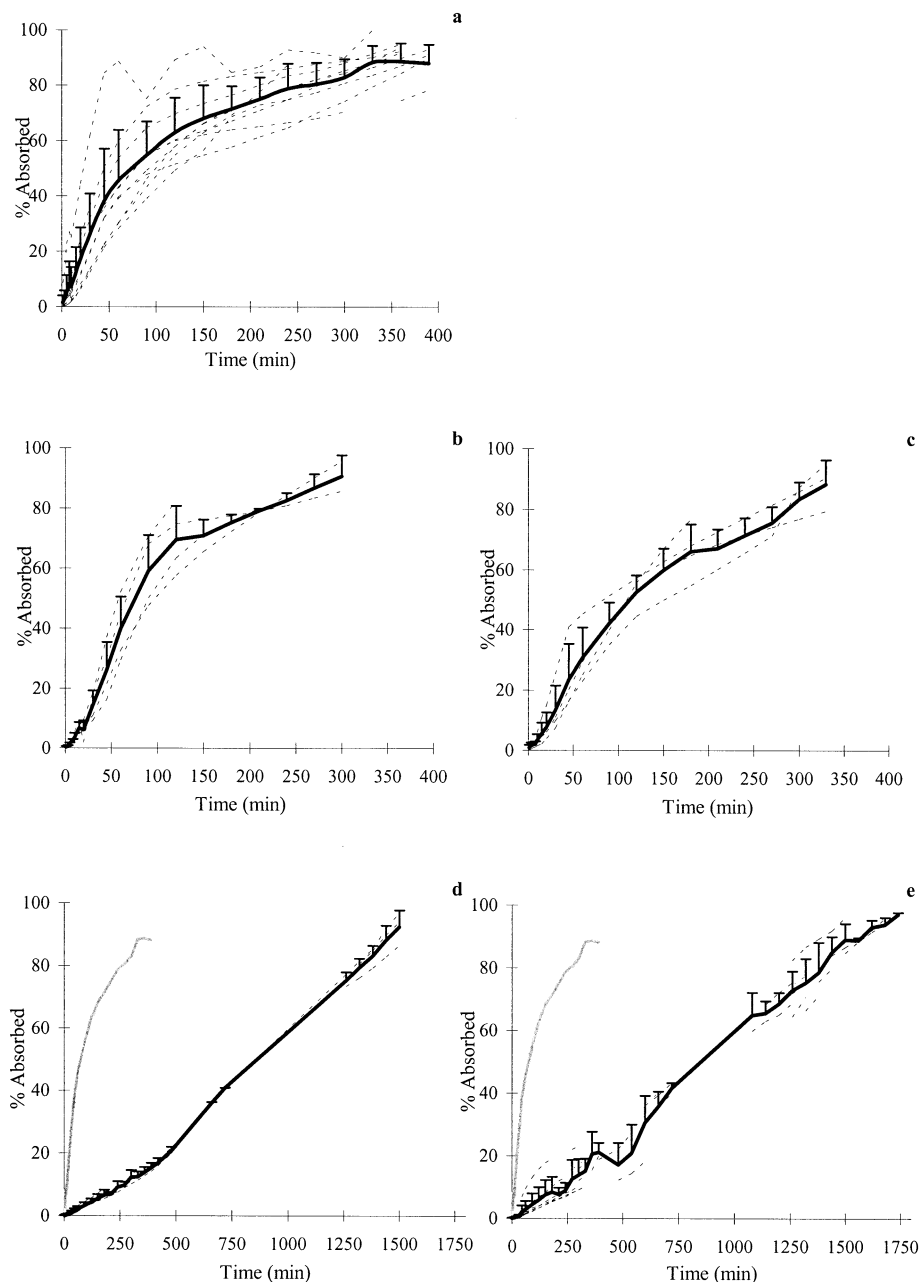

Fig. 4. Individual percent absorbed-time plots (dotted lines) and mean ± S.D. absorption profile (full line) of bupivacaine following brachial plexus injection in sheep of: (a) 75 mg bupivacaine HCl solution ($n = 10$); (b) 75 mg bupivacaine HCl solution with 75 μg epinephrine ($n = 4$); (c) 75 mg bupivacaine HCl solution with 150 μg epinephrine ($n = 4$); (d) 750 mg bupivacaine-loaded microspheres with a drug-RG503H polymer ratio of 40–60 ($n = 3$); (e) 750 mg bupivacaine-loaded microspheres with a drug–RG503H polymer ratio of 50–50 ($n = 8$). In d and e, the grey line represents the mean absorption profile following administration of 75 mg bupivacaine HCl solution 75 mg (a). Bupivacaine doses are expressed in bupivacaine HCl equivalent.

Table 3
Biopharmaceutic parameters of bupivacaine following brachial administration in sheep of 75 mg bupivacaine HCl solution: bupivacaine alone ($n=10$), bupivacaine and 75 μg epinephrine ($n=4$), bupivacaine and 150 μg epinephrine ($n=4$), and bupivacaine-loaded microspheres (750 mg of bupivacaine) with a ratio drug–PLGA of 40–60 ($n=3$), and of 50–50 ($n=8$)

Formulation/dose		Sheep	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2\beta}$ (h)	$T_{10\%}$ (h)	$T_{50\%}$ (h)	$T_{90\%}$ (h)
Bupivacaine HCl solution (75 mg)	Alone	S1	80	0.8	6.3	0.4	2.0	6.4
		S5	119	0.8	1.6	0.3	1.3	5.0
		S6	99	1.5	5.5	0.4	1.7	5.6
		S7	128	0.3	4.5	0.2	0.7	5.5
		S8	126	0.8	2.2	0.3	1.5	5.8
		S9	59	0.8	1.9	0.4	1.8	4.1
		S10	166	0.8	9.0	0.3	1.8	6.5
		S11	38	0.2	9.6	0.2	1.3	8.0
		S12	93	0.3	8.4	0.2	0.9	5.3
		S13	226	0.5	7.7	0.2	1.3	6.0
		Mean	113	0.7	5.7	0.3	1.4	5.8
		±S.D.	54	0.4	3.0	0.1	0.4	1.0
	+Epinephrine 75 μg	S6	75	0.8	6.0	0.5	1.6	4.5
		S10	109	0.8	1.1	0.3	1.0	2.3
		S11	59	1.0	2.1	0.5	1.5	4.7
		S12	307	1.0	7.1	0.3	1.1	5.8
		Mean	138	0.9	4.1	0.4	1.3	4.3
		±S.D.	115	0.1	2.9	0.1	0.3	1.5
	+Epinephrine 150 μg	S3	139	0.8	6.2	0.2	1.4	5.5
		S7	88	1.0	4.9	0.5	1.9	7.4
		S12	51	1.5	8.5	0.5	2.5	0.2
		S13	74	0.8	3.5	0.6	1.8	0.7
		Mean	88	1.0	5.8	0.4	1.9	5.4
		±S.D.	37	0.4	2.1	0.1	0.5	1.5
Bupivacaine HCl microspheres (750 mg)	Drug–PLGA 40–60	S6	65	6.5		5.0	14.3	24.1
		S10	75	6.0		4.3	14.3	23.7
		S11	74	5.0		3.5	14.4	25.9
		Mean	71	5.8		4.3	14.3	24.6
		±S.D.	6	0.8		0.7	0.1	1.2
	Drug–PLGA 50–50	S1	174	7.0		4.8	14.2	26.5
		S2	178	7.0		4.4	13.6	23.3
		S3	45	10.0		5.4	16.2	27.4
		S4	101	1.5		1.1	13.9	26.4
		S7	58	4.5		3.1	13.9	25.9
		S9	132	10.0		6.0	16.1	25.3
		S11	188	7.0		2.9	15.8	27.7
		S12	126	5.5		4.3	16.2	26.6
		Mean	125	6.6		4.0	15.0	26.1
		±S.D.	54	2.8		1.6	1.2	1.4

Bupivacaine doses are expressed in Bupivacaine HCl equivalent.

increase in plasma concentrations between 3–6 and 24 h postdosing with a subsequent decrease. Such plasma profiles suggested that this micro-sphere formulation did not allow an in vivo constant and sustained release of bupivacaine, although a controlled release existed as illustrated by the late C_{max} (24 h).

The evaluation of the pharmacodynamic effect of bupivacaine-loaded microspheres showed an 8-fold increase in the duration of motor blockade in comparison with bupivacaine given as a solution (Estébe et al., 2001). Such increase in duration of action was consistent with the in vivo absorption kinetics. It should be noticed that this increase in duration of action is obtained with a 10-fold higher dose of bupivacaine loaded in microspheres compared to the dose of bupivacaine in solution. However, despite the 10-fold higher dose, the maximal plasma levels were similar. Such feature is much interesting given that the systemic toxicity of local anesthetics is related to maximal plasma levels precluding the use of high doses of these drugs when administered as solutions.

A further increase in the duration of action may be of interest in some postsurgical situations. Such an increase could be obtained by incorporation of dexamethasone in the microsphere formulation as shown recently in animal models different from our model (Castillo et al., 1996; Dräger et al., 1998). The mechanism(s) by which dexamethasone increases the duration of the blockade is yet unclear but does not result from a modification in the pharmacokinetics of bupivacaine released from microspheres (Castillo et al., 1996). It could result from: (i) a direct action of dexamethasone on the nerves leading to an alteration of the functioning of ion channels; (ii) a local modification of the mononuclear and polymorphonuclear leukocyte foreign-body response to the microspheres; (iii) an inhibition of the local anesthetic tachyphylaxis. Work is currently in progress using our brachial plexus model in sheep to investigate the influence of dexamethasone co-encapsulation with bupivacaine in PLGA microspheres. Improvement in pharmacodynamics may arise from new drug delivery systems. Indeed, a report by Kohane et al. (2000) on lipid–protein–sugar particles containing bupivacaine showed that this drug delivery system favored the sensory blockade towards the motor blockade in a sciatic nerve block model in rats. Such feature is interesting given that motor blockade is not warranted in all clinical situations and should be confirmed in other models.

In conclusion, bupivacaine-loaded PLGA microspheres were successfully prepared with a spray-drying method. The results after administration of bupivacaine–PLGA RG 503H microspheres near the brachial plexus in sheep, in accordance with the in vitro release studies, showed: (i) a controlled release of the local anesthetic at the injection site, avoiding high plasma concentrations; and (ii) a zero-order absorption profile in accordance with a prolonged pharmacodynamic effect of the local anesthetic over 24 h (Estébe et al., 2001). Therefore, on the basis of the current work, performed in an animal model comparable in body weight and length of nerves to adult humans, such a drug delivery system should be of interest to improve the therapeutic index of bupivacaine which is largely used for regional anesthesia and for regional management of pain following upper limb surgery.

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