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Research paper

Epidural, intrathecal and plasma pharmacokinetic study of epidural ropivacaine in PLGA-microspheres in sheep model

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ABSTRACT

Background: Microparticulate local anesthetics-loaded delivery systems are known to provide a controlled release of drug and to reduce systemic toxicity resulting from transient high plasma concentrations. The aim of this study was to evaluate epidural, intrathecal and plasma pharmacokinetics of ropivacaine following epidural administrations of repeated boluses or infusions and to compare them with the epidural administration of polylactide-co-glycolide ropivacaine-loaded microspheres.
Methods: In the first step, the epidural and intrathecal pharmacokinetics was evaluated in 3 Lacaunes ewes, receiving epidural continuous infusion of ropivacaine with increasing doses (20, 50 and 100 mg/h). Then, six animals received an epidural administration of ropivacaine-loaded microspheres (500 mg), three others received ropivacaine in epidural bolus (30 mg) followed by infusion (2 mg/ml during 6 h), and the last three animals received three successive epidural boluses of ropivacaine (50 mg) at 2 h interval. A simultaneous microdialysis technique was used to measure epidural and intrathecal concentrations of ropivacaine.
Results: After epidural administration of ropivacaine-loaded microspheres, Cmax in plasma was around 100 ng/ml while epidural and intrathecal Cmax of ropivacaine were close to 600 and 150 μg/ml, respectively. The ratios of intrathecal to epidural AUC (AUCit/AUCepi) for bolus administration, bolus + infusion administration, and for microspheres were 13.4 ± 2.4; 14.1 ± 6.1 and 33.9 ± 22.6%, respectively. This suggested that administration of ropivacaine as microspheres increased the transmeningeal passage of ropivacaine in comparison to other administration regimens.
Conclusions: Epidural administration of ropivacaine-loaded microspheres led to the sustained levels of ropivacaine in the intrathecal space compared to the boluses of ropivacaine solution. Moreover, epidural administration of microspheres resulted in the highest efficiency in intrathecal uptake of ropivacaine compared to administration in solution.

1. Introduction

Epidural administration of local anesthetics is a common practice in anesthesia and for the treatment of postoperative pain. Among the drugs used by epidural route, ropivacaine and bupivacaine are the drugs of choice. Their potency and duration of action are similar but ropivacaine has been shown to be less cardiotoxic and less neurotoxic than bupivacaine [1,2].

The fate of drugs administered by epidural route results from three competitive processes [3–5]: transmeningeal uptake into the intrathecal space, distribution into the epidural fat and uptake into the systemic circulation. The limiting factors for clinical use of epidural ropivacaine are a relatively short duration of action, and a high and rapid systemic absorption, resulting in a systemic toxicity and in a low intrathecal bioavailability (around 10%) [6].

The improvement of epidural administration of local anesthetics has been largely investigated in preclinical and clinical studies, either by adding other analgesic drugs, by using different administration regimens and by using controlled drug delivery systems.

In a sheep model, the co-administration of epinephrine was shown as an efficient way to increase ropivacaine and bupivacaine concentrations in intrathecal space [7]. The addition of epinephrine also decreased plasma levels of levobupivacaine and improved intraoperative anesthetic quality [8]. Additive agents such as

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epidural clonidine [9–11], morphine [12] or fentanyl [13] are known to provide a significant improvement in postoperative analgesia compared to local anesthetics alone.

Administrations of local anesthetics through indwelling epidural catheters present another commonly used technique. Different administration regimens were largely investigated in clinical studies: administration of sequential epidural boluses was compared to continuous infusions [14–16], and patient controlled epidural analgesia (PCEA) was tested with and without a continuous infusion [17,18]. In almost all cases, efficacy and toxicity results showed no significant interest of background epidural infusion, and a better anesthesia was obtained with sequential boluses compared to continuous infusion.

Controlled delivery systems offer an interesting alternative to epidural administration of local anesthetics leading to an increased amount of drug available in the area of interest and prolonging its duration of action. Many preclinical studies using epidural microspheres [19] or epidural formulations of liposomes [20,21] demonstrated a prolonged pharmacodynamic action with long-lasting constant plasma levels of local anesthetics. Such sustained-release systems could replace indwelling catheters for repeated injections or infusion, enhancing the patient compliance and convenience of administration. However, to our knowledge there is no study on the epidural and intrathecal biodistribution of local anesthetics after administration as drug delivery systems. Such studies are required to better understand the input of these drug delivery systems and to allow clinical development. The PLGA polymers have been used for microspheres fabrication because they are biocompatible and biodegradable. Indeed, they have already been used for medical applications, such as controlled formulation of drugs and orthopedic devices.

The purpose of this work was to compare the intrathecal uptake of ropivacaine after epidural administration of ropivacaine-loaded polylactide-co-glycolide (PLGA) microspheres to those obtained with clinically used analgesia regimens, i.e. successive boluses and bolus followed by a continuous epidural infusion.

2. Materials and methods

2.1. Materials

Polylactide-co-glycolide polymer (PLGA) Resomer RG503H (50 D,L-lactide:50 glycolide, M.W. 34 kDa) was supplied from Boehringer Ingelheim (Saint Germain-en-Laye, France).

Ropivacaine, bupivacaine and etidocaine (Astra-Zeneca, Paris, France) were used as substance of interest, internal standard of microdialysis and internal standard of HPLC, respectively. A Ringer's solution was used as perfusion fluid for microdialysis. All other reagents were of analytical grade.

2.2. Preparation of microspheres

Ropivacaine was encapsulated in microspheres as the base form obtained by precipitation in an alkaline medium (ammonium hydroxide) from a saturated aqueous solution of ropivacaine hydrochloride form.

Ropivacaine-loaded microspheres were prepared by spray-drying method. Ropivacaine base and polymer were dissolved in methylene chloride 2% (w/v) with different ropivacaine-polymer weight ratios: 40–60, 45–55, 47–53, and 50–50. The solution was spray-dried with a Mini Büchi B-191 laboratory spray-dryer (Büchi Laboratorium AG, Switzerland). 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 l/h).

Ropivacaine microspheres for *in vivo* administration were suspended in 15 ml of an aqueous solution containing 5% mannitol and 0.05% Tween 20 using ultrasonication for 2 min, then they were rapidly frozen (−40 °C). Microspheres were then freeze-dried for 48 h and kept at 4 °C until administration. Immediately before injection, microspheres were suspended in sterile water.

2.2.1. Microspheres characterisation

Size distribution of microspheres was assessed by laser light scattering using a Malvern Mastersizer S (Malvern Instruments, Orsay, France) after dispersion in 5 ml of a 0.05% Tween 20 aqueous solution and ultrasonication for 30 s. 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). Microspheres morphology and surface characteristics were observed by scanning electron microscopy (SEM) using a Jeol JSM Model 6400 electron micrograph (Jeol, Tokyo, Japan).

The drug-content of microspheres was evaluated by a HPLC system composed of a Waters Model 600 pump, a Waters Model 717 automatic injector (Waters Assoc., Milford, MA, USA) equipped with a spectromonitor Model 3100 UV detector set at 205 nm (Milton Roy, Riviera Beach, Floride, USA) and a data acquisition system Waters Empower-Pro. The analytical chromatographic column was a Lichrocart–Lichrospher RP-B Merck cartridge (length 125 mm, internal diameter 3 mm). The flow rate was 0.5 ml/min, and the temperature was maintained at 30 °C. The mobile phase consisted of a mixture of acetonitrile and pH 2.1, 0.01 M sodium dihydrogenphosphate (23:77). The weighted samples of ropivacaine-loaded microspheres (around 20 mg) were first dissolved in methylene chloride (1 ml). Then, the drug was extracted in 0.05 M sulphuric acid (5 ml) loaded with etidocaine (1 mg) as internal standard. After shaking (5 min) and centrifugation (3500*g*, 10 min), 50 μl of the aqueous phase was diluted in 2 ml of the mobile phase, and 20 μl of this diluted solution was injected into the chromatograph. The recovery of extraction was 86.4 ± 7.8 (n = 10).

2.2.2. In vitro release study

Release studies were performed with a Distek dissolution test system Model 5100A (North Brunswick, NJ) using a rotating paddle apparatus. The release medium was 0.2% NaCl and 1 mM HCl aqueous solution (890 ml, pH 2.5) thermostated at 37 °C. Weighted amount of ropivacaine-loaded microspheres (around 20 mg) was suspended in 10 ml of an aqueous solution containing 0.05% Tween 20, and then dropped in the release medium. Cumulative absorbance of ropivacaine solution was measured continuously at 205 nm by a Uvikon spectrophotometer Kontron Model 922 (St Quentin Yvelines, France) and processed as cumulative percent released using the Icalis Data System IDIS EE software (Berkshire, UK). Each batch of microspheres was analyzed six times.

2.3. Microdialysis and in vivo evaluation

Microdialysis was performed using a CMA/102 microinjection pump coupled to a microdialysis probe CMA/20 (membrane length of 10 mm, shaft length 140 mm, outer diameter 0.5 mm and molecular weight cut-off 20 kDa). The microdialysis probes were perfused at 1 μl min^{-1} with a solution of bupivacaine hydrochloride 1 mg ml^{-1} in a Ringer solution, pH 6.8.

Dialysates were collected by dilution in vials containing 100 or 200 μl (for intrathecal and epidural dialysates, respectively) solution of 1 μg ml^{-1} etidocaine using a CMA 142 microfraction collector (CMA Microdialysis, Solna, Sweden). A collection interval of 1 min during the first 15 min of the experiment and of 5 min during the further experiment allowed the sampling of 1 and 5 μl of dialysate, respectively.

After probe insertion in the intrathecal and epidural spaces, an *in vivo* equilibration with determination of relative loss (RL) of bupivacaine (n = 10 for each probe tested) was achieved over a period of 45 min. Throughout the experiments, the RL of bupivacaine was determined in each sample and was used to correct the dialysate concentrations [6].

Before and after *in vivo* implantation, the probes were tested *in vitro* in order to verify the lack of significant deterioration by comparison of RL bupivacaine. The inter-batch variability among microdialysis probes was low. The in vitro RL of bupivacaine checked before *in vivo* implantation was 0.44 ± 0.05 (n = 16).

The *in vivo* study was performed according to a protocol approved by the Local Committee of Laboratory Investigation and Animal Care of our institution, and was achieved in accordance with the rules and guidelines concerning the care and the use for laboratory animal experiments (agreement No. B35-238-21). The animals were 15 non-pregnant Lacaunes ewes, with a mean age of 2 ± 0.5 years and with a mean weight of 57 ± 5 kg. In the preliminary study, three animals received an epidural continuous infusion (6 h) of ropivacaine with increasing range of doses. Ropivacaine was infused at a rate of 10 ml/h during 2 h with the following concentrations: 2, 5 and 10 mg/ml. In the main experiment, the 12 animals were divided into three different groups: six received ropivacaine-loaded microspheres (500 mg/15 ml), three received a bolus of ropivacaine (30 mg/15 ml) followed by a 6h-infusion of ropivacaine (2 mg/ml, 10 ml/h), and the last 3 animals received three successive boluses of ropivacaine (50 mg/15 ml) separated by 2 h interval.

All the animals were sedated with intravenous thiopental and intubated for ventilation with isoflurane. After blunt dissection, a laminectomy was performed at L5–L6 level allowing the introduction of epidural catheter (external diameter of 2.0 mm and length of 120 mm) and epidural and intrathecal microdialysis probes in intrathecal and epidural spaces. An intravenous catheter was inserted in the jugular vein for blood sampling and administration of maintenance fluid. Hemodynamic parameters (ECG, invasive arterial blood pressure) were continuously monitored. An oesophageal thermistor was used to control the sheep body temperature, and a mobile heater was used to maintain body temperature above 37.5 °C.

A microdialysis sampling during 1 min was achieved every minute for 15 min and during 5 min to the end of each experiment. Blood samplings were collected at 0, 1, 2, 3, 5, 8, 10, 15, 20, 30, and then every 15 min to the end of the experiment (8 h for microspheres and bolus with infusion administration and 2 h for each successive boluses administration). After blood centrifugation (10 min at 3000*g*), plasma samples were kept frozen (−18 °C) until analysis.

At the end of the experiment, the animals were euthanized with intravenous injection of thiopental, potassium chloride and pancuronium.

2.4. Dialysates and plasma determination of ropivacaine

Ropivacaine concentrations in the intrathecal or epidural dialysates, and in plasma samples were determined using a HPLC method in the same conditions as described for drug-content in microspheres. Aliquots of 20 or 50 μL (for intrathecal or epidural samples, respectively) of the dialysate dilutions were immediately injected onto the chromatographic system. From plasma, ropivacaine was extracted according to a previously published method with slight modifications [22]. Briefly, 0.5 mL of plasma sample was alkalinized by 50 μL of 1 M NaOH and 3 mL of *n*-Heptane was added, after horizontal shaking (3 min) and centrifugation (3 min at 3500*g*), the organic phase was transferred to a conical vial containing 50 μL of 0.05 M H_2SO_4. After similar shaking and centrifugation, the organic phase was discarded, and the aqueous phase was buffered with 10 μL of 0.5 M K_2HPO_4 and 40 μL was injected onto the chromatographic system.

2.5. Data analysis

A non-compartmental analysis using the software package WinNonlin Pro (Pharsight, USA) was applied to epidural, intrathecal and plasma concentrations after administration of successive epidural boluses, and a compartmental analysis was applied to the epidural concentrations after bolus followed by continuous epidural infusion. The peak concentration (Cmax) and the corresponding time to peak concentration (Tmax) are derived from the raw data. The calculated parameters are AUCinf (area under concentration–time curves calculated to infinity), Vss (volume of distribution at steady state), CL and CLss (clearance and clearance at steady state), T1/2β (elimination half-life). Differences in pharmacokinetic parameters between sequential boluses were analyzed with an ANOVA test. A p-value less than 0.05 was considered as statistically significant.

Individual epidural release-time plots of ropivacaine after microspheres administration were estimated from deconvolution analysis using epidural elimination parameters obtained after solution administration. The percentage released is expressed as the fraction released.

3. Results and discussion

3.1. ***In vitro*** *studies*

Four different formulations of PLGA ropivacaine-loaded microspheres were prepared and evaluated *in vitro*. The experimental drug-content values were close to the theoretical drug-content values indicating high efficacy of entrapment of ropivacaine in PLGA-microspheres i.e. 99.8 ± 0.8% (Table 1). As previously shown with bupivacaine-loaded microspheres [23], the *in vitro* release kinetic of ropivacaine displayed significant differences related to the ropivacaine-polymer ratio (Fig. 1). All formulations displayed a biphasic profile with an initial burst, followed by a slow release phase. The maximum amount of ropivacaine released from drug-loaded microspheres and the percentage of the drug released in initial burst were higher when the ropivacaine-polymer ratio was high. The formulation with 45–55 ratio was chosen for the *in vivo* studies according to its interesting release profile, i.e. a relatively small burst (25%) and a 60% release of ropivacaine in 24 h. In a scanning electron micrograph, these microspheres presented smooth and regular spherical shape (Fig. 2). A mean diameter of these microspheres measured by laser light scattering was 5.2 μm (Table 1 and Fig. 3).

Table 1
Release kinetic parameters *in vitro* (T10%, T50%) and size parameters (D(v;0.5); D(4.3) and Span) of ropivacaine-loaded PLGA-microspheres.

Drug–polymer ratio	Experimental drug-content (%)	T10% (h)	T50% (h)	D(v;0.5) (μm)	D(4.3) (μm)	Span
50/50	49.4	0.8	0.8	3.36	5.44	3.6
45/55	44.9	0.8	15	3.57	5.2	3.4
47/53	47.9	0.8	22	3.82	5.69	3.4
40/60	41.6	0.8	23	4.42	5.81	2.8

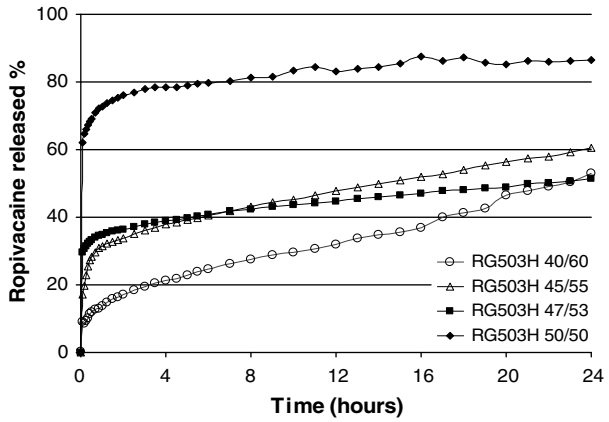

Fig. 1. *In vitro* release profile of ropivacaine from PLGA RG503H microspheres with different drug–polymer ratios.

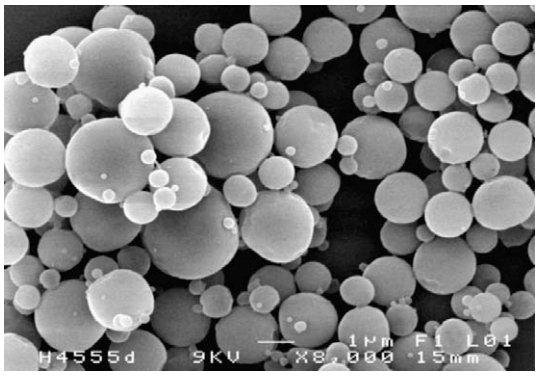

Fig. 2. Scanning-electron microscopy of ropivacaine-loaded microspheres prepared with PLGA RG503H polymer with drug–polymer ratio (%): 45–55.

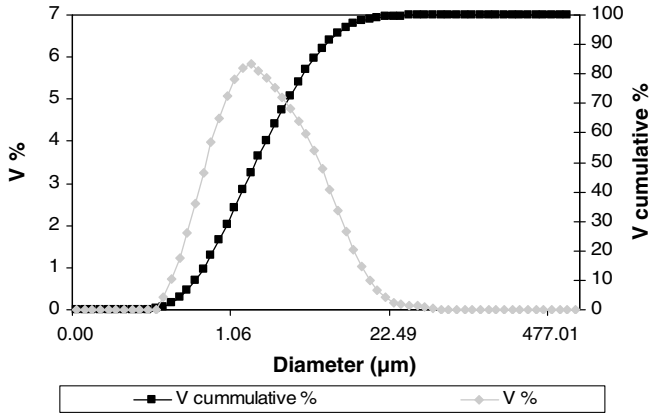

Fig. 3. Plot of size distribution for 45/55 RG503H ropivacaine-loaded microspheres.

3.2. In vivo study

This study was designed in a sheep model using microdialysis allowing frequent sampling with minimal physiological perturbations. Simultaneous analysis of local anesthetic concentrations in epidural and intrathecal spaces by microdialysis technique allowed us to follow the unbound concentrations, i.e. the pharmacologically active fraction of local anesthetics at the site of injection and in the vicinity of the site of action, in the intrathecal space.

3.2.1. Linear pharmacokinetics after continuous infusion with increasing doses of ropivacaine

Concentrations in epidural and intrathecal spaces and in plasma after epidural infusion of increasing doses of ropivacaine are presented in Fig. 4. In epidural space, at the site of injection, concentration profiles displayed three successive plateaus at different levels (about 1440, 3410, 6680 µg/ml for 20, 50 and 100 mg/h, respectively). The epidural concentrations at steady state (Css) as the function of infusion rate (k_0) are presented in Fig. 5. Css increases linearly ($r^2 = 0.9654$) with increasing k_0. Thus, the epidural clearance CLss ($n = 3$) was similar and independent of the administration rate (20, 50 and 100 mg/h) 0.194 ± 0.016, 0.207 ± 0.033, and 0.206 ± 0.020 ml/min, respectively, suggesting linear pharmacokinetics. The measured concentrations in intrathecal space revealed a high variability and did not present plateaus as a function of the increase of k_0, as in epidural space. The uptake of ropivacaine through meninges into intrathecal space was delayed and was not clearly dose-dependent. The systemic concentrations of ropivacaine increased progressively during the study period (Fig. 4C). These observations should be considered for clinical practice, because the raising concentrations in epidural space apparently did not influence the concentrations in the cerebrospinal fluid (CSF), but led to an increase in plasma concentrations, directly related to local anesthetics toxicity. Hence, the prediction of intrathecal concentrations is difficult using epidural or plasma data.

These results raised the question of the influence of both drug concentration and volume injected to obtain an efficient analgesia. Recently, no significant differences on analgesia and motor blockade were reported after different doses of epidural bolus of ropivacaine with fentanyl [24]. This is in accordance with our results demonstrating only a low increase of intrathecal concentrations after increase in concentrations of drug infused. After epidural administration of sequential boluses, it was demonstrated that low-concentrations/high-volumes of ropivacaine and fentanyl [25] and of bupivacaine [26] were as efficient for sensory blockage as administration of high-concentrations/low-volumes. However, the administration of low-concentration/high volumes provided a significant decrease in total drug used, suggesting that the therapeutic index of administrated drugs was widened if a low-concentration/high-volume solution was used. In the current study, we investigated only different infusion concentrations of ropivacaine given in a continuous infusion, but it could be of interest to investigate also the influence of different volumes of ropivacaine on intrathecal concentrations.

3.2.2. Sequential boluses

The epidural administration of ropivacaine in three sequential boluses resulted in the highest epidural Cmax whatever the administration mode, i. e. for the first bolus 2268 ± 819 µg/ml, Fig. 6A. In the epidural space, pharmacokinetic parameters were characterised by a high variability between individuals and between sequential injections (Table 2). The epidural clearance after epidural administration was of the same range as in the previous studies in sheep [6,7] between 0.8 and 0.5 ml/min. In the intrathecal space, the AUCinf after the first bolus was 12565 ± 2184 min µg/ml, after the second 19021 ± 6855 min µg/ml, and finally after the third attained 21744 ± 6518 min µg/ml (Table 3). This non-significant increase of AUC ($p = 0.2$) was due to the intrathecal residual concentrations of ropivacaine obtained with the previous administrations (significant increase in Cmax). The plasma pharmacokinetic parameters were highly variable. The plasma AUCinf was increasing with successive epidural boluses as in the intrathecal space, but their increase should probably be related to a systemic phenomenon (variation in clearance or distribution) since the plasma elimination half-life was increased.

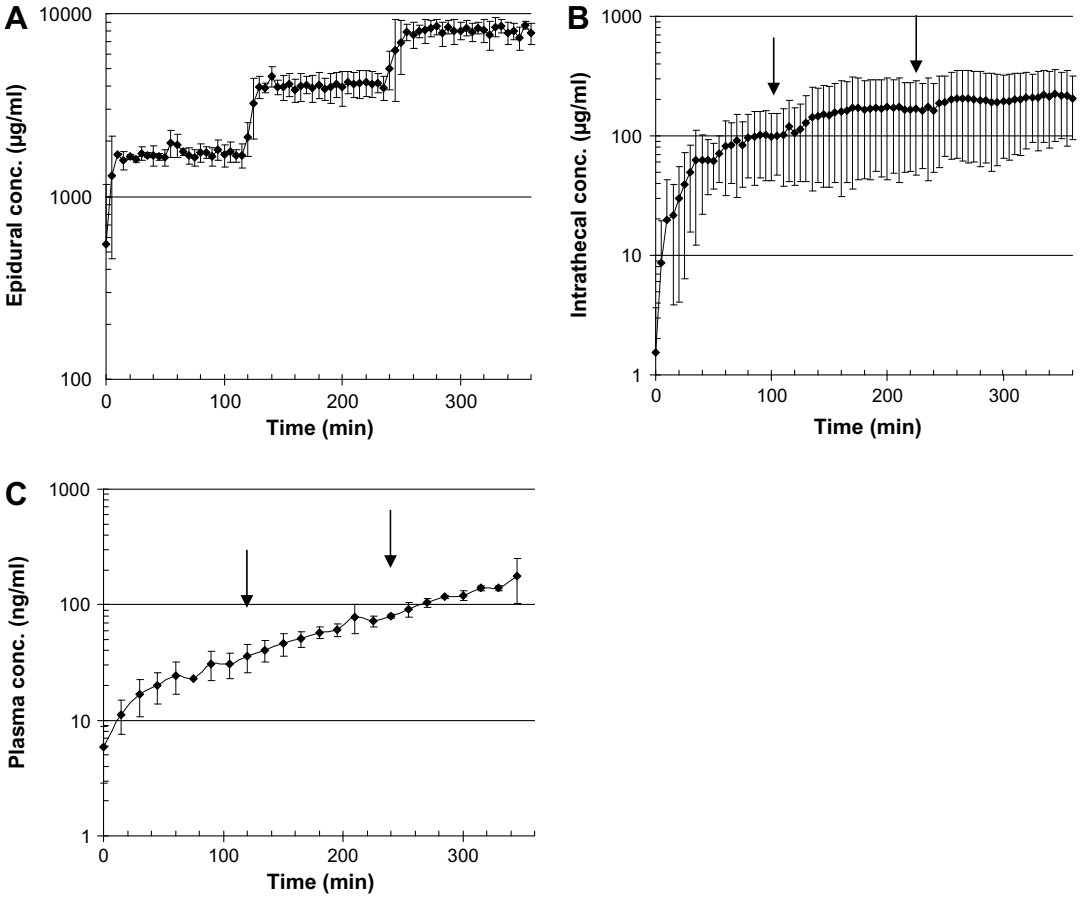

Fig. 4. Concentrations of ropivacaine in (A) epidural space, (B) intrathecal space and (C) in plasma, after epidural 2 h continuous infusion of ropivacaine at 20, 50 and 100 mg/h. Arrows on intrathecal and plasma graphs represent the change in infusion rate.

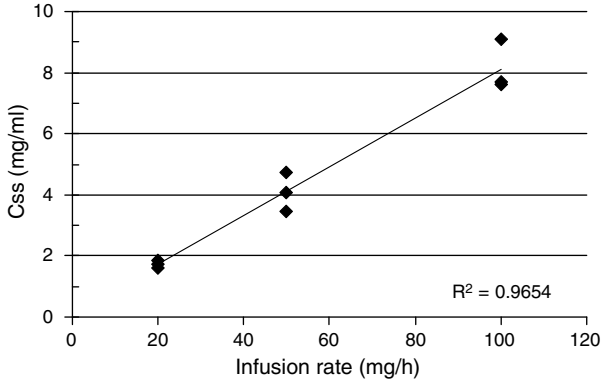

Fig. 5. Epidural concentrations at steady state after epidural infusion: 20, 50 and 100 mg/h, during 2 h.

3.2.3. Bolus followed by continuous infusion of ropivacaine

The concentration–time profiles of ropivacaine after epidural administration of bolus followed by infusion are shown in Fig. 6. The epidural CLss (0.254 ± 0.032 ml/min) obtained with a monocompartmental analysis was slightly higher than that calculated in a dose ranging study with the same rate of administration of ropivacaine (0.194 ± 0.016 ml/min). The reason for such an increase is unknown, since the only difference between both administrations was the initial bolus, which should not influence the CLss. The epidural T1/2β after bolus + infusion administration was similar as after first sequential bolus injection i.e. 70 ± 4 min *vs* 68 ± 16, respectively.

The epidural Cmax of ropivacaine after bolus followed by infusion administration was lower than those after sequential boluses administration (1533 ± 288 µg/ml *vs* 2268 ± 819 µg/ml, respectively). In the intrathecal space, a Cmax of ropivacaine after bolus + infusion administration was higher than those after administration of sequential boluses (Fig. 6B). A concentration level similar to bolus followed by infusion was reached only after the third sequential bolus administration (136 ± 21 µg/ml).

3.2.4. Ropivacaine microspheres

The epidural concentrations of ropivacaine after epidural administration of PLGA ropivacaine-loaded microspheres (500 mg) are shown in Fig. 6A. The epidural Cmax of 597 ± 249 µg/ml was reached 20 min after administration. During first 2.5 h after administration, the epidural profile showed absorption and distribution phases followed by a plateau corresponding to the apparent elimination phase. This profile of ropivacaine epidural concentrations could be related to the biphasic kinetics of the release of ropivacaine from microspheres (Fig. 7) displaying an initial rapid release phase (about 2.0–2.5 h) followed by a zero-order release. Such a profile induced by the microspheres formulation is interesting since it mimics the bolus + infusion administration. The amount of ropivacaine released immediately as a burst (*in vitro* study) corresponds to the bolus, and the following zero-order release phase corresponds to the infusion.

The controlled release of ropivacaine in the epidural space significantly decreased the elimination rate indicating that the

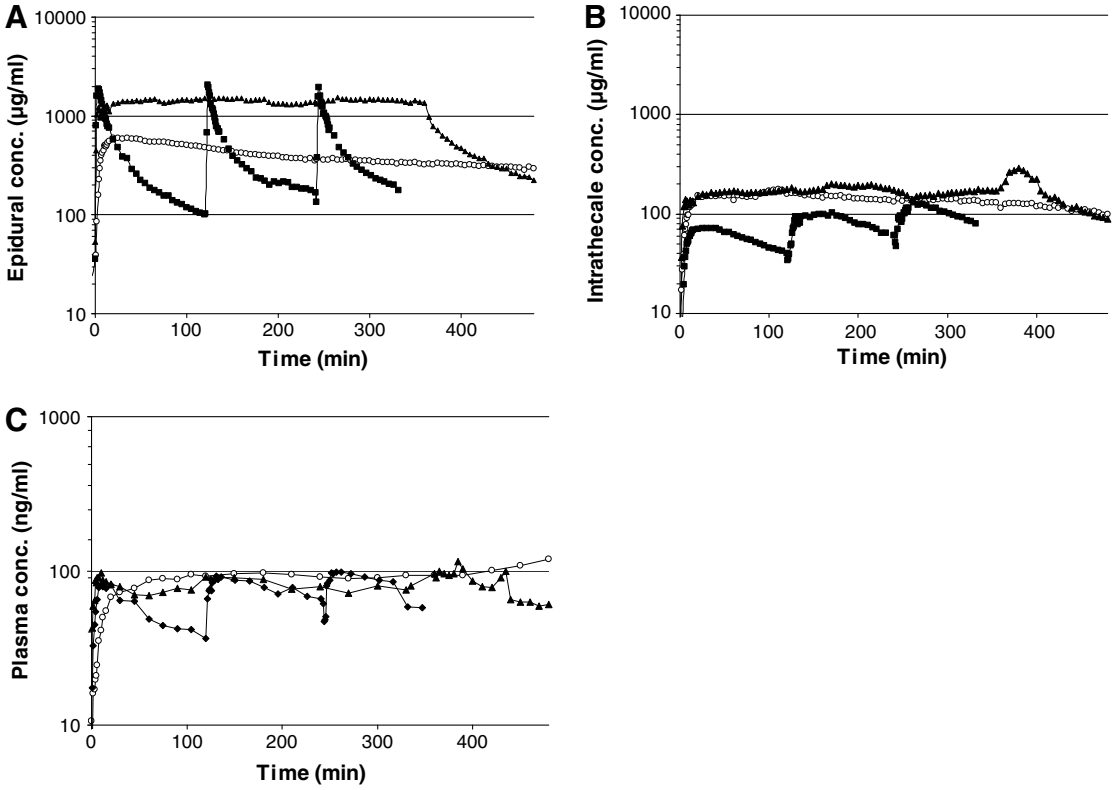

Fig. 6. Mean concentrations of ropivacaine in (A) epidural space, (B) intrathecal space and (C) in plasma, after epidural administration of ropivacaine-loaded microspheres (500 mg): open circles, bolus followed by continuous infusion (150 mg): full triangles and three sequential boluses (150 mg): full squares.

Table 2
Epidural pharmacokinetic parameters of ropivacaine after epidural administration in three successive boluses (non-compartmental analysis).

	Bolus 1 (50 mg)	Bolus 2 (50 mg)	Bolus 3 (50 mg)
AUC inf (min µg/ml)	64786 ± 21733	101290 ± 32649	82675 ± 24401
Cmax (µg/ml)	2268 ± 819	2696 ± 745	2813 ± 303
Tmax (min)	3.0 ± 1.0	2.3 ± 0.6	4.0 ± 1.7
T1/2 β (min)	68 ± 16	123 ± 60	62 ± 22
Cl (ml/min)	0.8 ± 0.3	0.5 ± 0.2	0.6 ± 0.2
Vss (ml)	52 ± 14	65 ± 11	37 ± 15

elimination was rate limited by the release from microspheres. Such a feature was also observed in the intrathecal space suggesting a constant input rate of ropivacaine into the intrathecal space.

In the intrathecal space, Cmax (201 ± 117 µg/ml) was reached, as in epidural space, about 20 min after epidural administration. During all the study period, the intrathecal concentrations were decreasing very slowly (Fig. 6B).

Despite that the epidural concentrations of ropivacaine after microspheres administration were almost threefold lower than

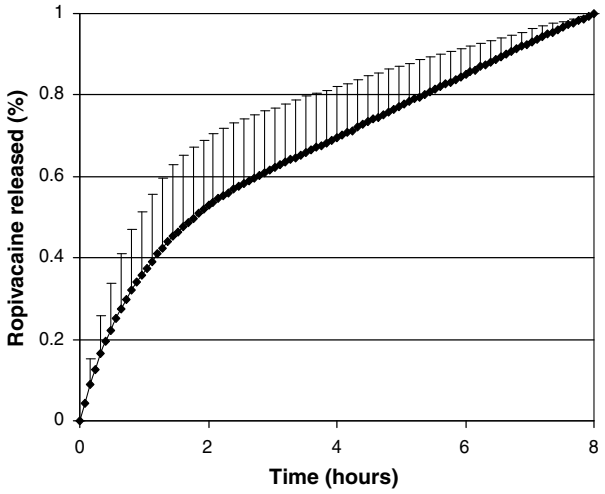

Fig. 7. *In vivo* mean release rate of ropivacaine from microspheres after epidural administration.

Table 3
Intrathecal and plasma parameters of ropivacaine after epidural administration in three successive boluses (non-compartmental analysis).

	Intrathecal space			Plasma		
	Bolus 1 (50 mg)	Bolus 2 (50 mg)	Bolus 3 (50 mg)	Bolus 1 (50 mg)	Bolus 2 (50 mg)	Bolus 3 (50 mg)
[*]AUCinf (min µg/ml)	12565 ± 2184	19021 ± 6855	21744 ± 6518	15958 ± 13542	40202 ± 41559	54508 ± 17333
[*]Cmax (µg/ml)	82 ± 8	116 ± 18[#]	136 ± 21[#]	85 ± 26	97 ± 57	102 ± 64
Tmax (min)	26 ± 11	36 ± 24	23 ± 21	24 ± 19	19 ± 23	12 ± 3
T1/2 β (min)	102 ± 25	99 ± 62	103 ± 44	148 ± 85	265 ± 159	446 ± 187

[*] In plasma the AUCinf is expressed in (min.ng/ml) and Cmax in (ng/ml).
[#] The value of Cmax for bolus 2 was significantly higher than for bolus 1 and Cmax for bolus 3 was significantly higher than for bolus 2.

Table 4
Ratio of intrathecal to epidural AUC of ropivacaine (expressed as %) after epidural administration in different modes of administration.

Ratio	Successive boluses			Infusion				
	1 50 mg	2 50 mg	3 50 mg	20 mg/h (2 heures)	50 mg/h (2 heures)	100 mg/h (2 heures)	Bolus+infusion 30 mg + 20 mg/h (6 heure)	Microspheres 500 mg
AUCit/AUCépi (%)	13.4 ± 2.4	22.8 ± 16.7	27.0 ± 11.0	4.2 ± 2.4	3.7 ± 2.5	2.5 ± 1.6	14.1 ± 6.1	33.9 ± 22.6

that after bolus followed by infusion administration, in the intrathecal space the concentrations of ropivacaine after both administrations were close (176 ± 132 and 201 ± 117 μg/ml, respectively). Such a feature suggests the enhancement of transmeningeal passage of ropivacaine after administration in microspheres.

Ropivacaine plasma Cmax was reached 1 h after epidural administration and plasma concentrations remained constant close to 100 ng/ml (1000 times lower than intrathecal concentrations), all over the study period (Fig. 6C).

Thus, ropivacaine-loaded microspheres can be an interesting drug formulation with advantages compared to patient controlled epidural anesthesia and continuous infusion, since the intrathecal pharmacokinetic profiles of ropivacaine obtained after a single bolus administration of microspheres are similar to those observed after continuous infusion. Moreover, high doses of local anesthetics loaded into microspheres did not increase plasma concentrations, becoming even more interesting with regard to the systemic toxicity which is related to their maximal plasma levels.

3.2.5. Transmeningeal uptake of ropivacaine

The intrathecal bioavailability of ropivacaine was found near 10% in our previous work [6]. In the current study, the intrathecal bioavailability of ropivacaine could not be directly calculated from the experimental data, because studied animal did not receive intrathecal administration of ropivacaine. Nevertheless, we can compare intrathecal and epidural AUC after different regimens of administration.

The ratio of intrathecal to epidural AUC (AUCit/AUCepi) of ropivacaine after continuous infusion was 4.2 ± 2.4. Moreover, this ratio decreased with increasing rate of infusion, suggesting that efficacy of intrathecal uptake was inversely proportional to the increase of infused concentrations (Table 4). This may suggest a saturation in the uptake through meninges as a result of the very high levels of concentrations (between 3 and 6 times the level attained with the other regimens of administration).

The ratios AUCit/AUCepi calculated for the first successive bolus and for bolus followed by infusion administration were very close: 13.4 ± 2.4 and 14.1 ± 6.1, respectively (Table 4). The ratio calculated for the second and third successive bolus administration was higher, which is not surprising taking into account the accumulation of concentrations in intrathecal space.

Finally, the highest AUCit/AUCepi ratio was obtained with microspheres administration: 33.9 ± 22.6, suggesting that this mode of administration provides the highest transmeningeal uptake of ropivacaine.

As mentioned before, the fate of drugs administrated by epidural route is driven by three competitive processes. The systemic uptake is the main process, as we showed recently [6] around 70% of the administrated dose of ropivacaine are directly and rapidly absorbed into the systemic circulation. Around 10% of the administrated dose cross the meninges and reach the intrathecal space. And finally, the last fraction of the drug is probably distributed into epidural fat in a reversible process. After administration as microspheres, there is a decrease in the rate of uptake of ropivacaine in the systemic circulation as shown by the Tmax (Tmax of around 20 min after bolus of solution compared to Tmax of around 1 h after microspheres). This decrease in the systemic absorption rate induced by the controlled release of drug from the microspheres led to an increase in intrathecal uptake. This feature is not unlikely since in the previous work [7], we had shown that a temporary decrease in systemic uptake of ropivacaine and bupivacaine (induced by a vasoconstriction) led to an increase in the intrathecal uptake. Hence, it appears that the intrathecal bioavailability of epidural drugs is highly dependent on the elimination via the systemic uptake. The increase in intrathecal uptake should not be related to a modification in meninges permeability since the characteristics of PLGA polymer are not known to modify the permeability through membranes. However, an increase in permeability could be obtained using polymers such as chitosan, which are known to improve the permeability through intestinal membrane [27,28]. This feature is explained by mucoadhesivity of chitosan, which results in increased time of contact with cell surface and by interaction of chitosan with cytoskeletal F-actin, which is associated with proteins in the tight junctions. Thus, chitosan microspheres stimulate the opening of tight junctions and increase paracellular permeability. A strategy using polymers enhancing membrane permeability should be investigated for a delivery of numerous therapeutic molecules, such as proteins, genes or hydrophobic drugs that are poorly transported across biological membranes.

4. Conclusion

In summary, sustained concentrations of ropivacaine were obtained in CSF after epidural administration in PLGA-microspheres. Compared to clinically used administrations, such as sequential boluses or bolus followed by infusion, the epidural administration of appropriate formulations of PLGA-microspheres of ropivacaine led to a reduced systemic absorption allowing higher drug uptake through meninges.

Therefore, on the basis of this work, a microparticulate system for epidural administration could become a useful therapeutic tool to overcome the problems of epidurally used drugs that have a low intrathecal bioavailability, a systemic toxicity resulting from transient toxic plasma concentrations or a relatively short duration of action.

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