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Prolongation of epidural bupivacaine effects with hyaluronic acid in rabbits

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Abstract

To assess the prolongation of epidural bupivacaine by hyaluronic acid viscous formulations we designed a cross-over study in rabbits. Different doses of bupivacaine (3 or 6 mg) either as a solution (bupivacaine hydrochloride), or as viscous formulations with hyaluronic acid (bupivacaine base and bupivacaine hydrochloride) were administered in a rabbit model of epidural anesthesia. In the first part of the study, in vitro release characteristics were determined. Then pharmacodynamic effects and pharmacokinetic profiles of each bupivacaine formulation were studied. The rank order release rate of bupivacaine in vitro was always hydrochloride solution $\gg$ viscous physical mixture of bupivacaine with hyaluronic acid $>$ viscous ionic complex of bupivacaine base with hyaluronic acid. Onset time of epidural anesthesia was similar whatever the formulation of bupivacaine used. We did not find any blockade prolongation when 3 mg bupivacaine was administered, but significant blockade prolongations were observed with viscous formulations incorporating 6 mg bupivacaine. The observed reduction in the absorption rate of bupivacaine into the systemic circulation for both viscous hyaluronic formulations after 6 mg of bupivacaine may explain the prolongation of spinal effects. Drug release and duration of action were found to be viscosity controlled as linear relationships were found between pharmacodynamic effects and viscosity. Our results were in accordance with those reported with bupivacaine–cyclodextrin complex, another formulation with a molecular dispersion of the drug, resulting in a moderate prolongation of action.

Keywords: Bupivacaine; Epidural anesthesia; Hyaluronic acid; Rabbits

1. Introduction

Epidural anesthesia is widely used during postoperative period. In addition to low pain scores and comfort, all techniques of regional anesthesia permit earlier rehabilitation and shorten the duration of hospitalization of patients (Capdevila et al., 1999). Unfortunately, duration of acute pain following surgery is often longer than duration of analgesia provided by epidural local anesthetics. So a catheter insertion is required for continuous epidural administration, but such procedure is associated with several side effects such as infection, or dislogding of catheter to intrathecal or vascular spaces.

Several attempts were performed to prolong the duration of local anesthetic effects such as modifications of molecular structures (Scurlock and Curtis, 1981); addition of adjuvants such as vasoconstrictors

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(Hassan et al., 1985a) or sodium bicarbonate (Racle et al., 1988); incorporation in microparticular drug delivery systems such as microspheres (Estebe et al., 1995; Malinovsky et al., 1995; Le Corre et al., 2002) or liposomes (Legros et al., 1990; Boogaerts et al., 1994; Mowat et al., 1996; Malinovsky et al., 1999); complexation with cyclodextrins (Freville et al., 1996; Dollo et al., 1998). Another way consists in an association with agents resulting in viscous formulations. Previous experimental data with dextrans (Bridenbaugh, 1976; Hassan et al., 1985a) or iophendylate (Langerman et al., 1991) added to local anesthetics showed significant prolongation of spinal analgesia duration. Incorporation of lidocaine in cellulosic gels showed a prolonged sciatic nerve block in animals (Paavola et al., 1995, 1998). However, these additives have immunogenic properties, and most of them have a neurotoxicological potency against structures of nervous system, so they appear not safe for spinal administration in humans.

In contrast, hyaluronic acid, a naturally occurring high molecular weight glycosaminoglycan composed of repeating disaccharides units of *N*-acetylglucosamine and D-glucuronic acid, has interesting properties, i.e. viscosity, lack of immunogenicity, biocompatibility (Doherty et al., 1994). Formulations of local anesthetics with hyaluronic acid of medium or high molecular weight induced prolonged analgesia following spinal administration in mouse (Hassan et al., 1985b) as well as epidural administration in rabbits (Doherty et al., 1995). Other experimental results with different local anesthetics were controversial, the prolongation of analgesia appears to depend upon the animal model used and the site of injection. Indeed mixtures of hyaluronic acid and lidocaine showed significant prolongation of epidural analgesia in rabbits (Doherty et al., 1995) but not in dogs (Doherty et al., 1996) and mixtures of hyaluronic acid and bupivacaine significantly increased the duration of infra-orbital nerve block in rats (Hassan et al., 1985b) but did not affect the duration of sensory nerve block following perineural administration in man (Johansson et al., 1985).

The aim of this study was to investigate the influence of the dose of bupivacaine administered epidurally when mixed with high molecular weight hyaluronic acid on the duration of analgesia in the rabbit, an animal model which has previously shown a prolongation of epidural lidocaine analgesia. In the first part, physical mixtures and ionic complexes were prepared by mixing hyaluronic acid with both bupivacaine hydrochloride and base forms and drug release characteristics from these formulations were obtained with an in vitro two-compartment model. Then we designed a cross-over study with two doses (3 or 6 mg) of bupivacaine base and bupivacaine hydrochloride mixed with hyaluronic acid and the pharmacokinetic profiles were compared in our rabbit model of epidural anesthesia (Malinovsky et al., 1997).

2. Materials and methods

2.1. Animals

Fifteen albino New Zealand rabbits weighting 2.5–2.7 kg were included in the study, which was performed in accordance with French Ministry of Agriculture laws and guidelines for laboratory animal experiments, and approved by our Institutional Animal Investigation Committee.

Rabbits were chronically instrumented as follows: under general anesthesia and sterile conditions an epidural catheter was inserted at the caudal level. After cutaneous incision of the tail, interspace between two caudal vertebrae was incised and a 23-gauge catheter (Periquick®, Gamida Lab., Eaubonne, France) was gently slided 10 cm cephalad into the epidural space in order to set the tip of the catheter at the L_6 level. The right position of the catheter was tested as previously described (Malinovsky et al., 1997), by epidural injection of 1 ml of 2% lidocaine allowing a complete motor and sensory blockade of the lower body for skin closure and insertion of an arterial catheter. The system was tunnelized and secured, and implanted subcutaneously on the back of the rabbit. The catheter was flushed with 0.2 ml of saline solution. Then, an arterial catheter was inserted via the femoral artery and heparinized.

After the epidural catheter had been inserted, rabbits were housed individually in standard cages with free access to food and water and with a natural light/dark cycle. They were included in the study the day after catheter implantation, only if they had a symetric walking and normal behavior, i.e. no allodynic reactions.

2.2. Preparation of injections

Bupivacaine hydrochloride (B) was obtained from Astra Pharmaceuticals (Astra Pain Control, Sodertalje, Sweden) and used to prepare 0.3% solutions which were sterile filtered (0.22 μm) under aseptic conditions. Bupivacaine base (Bb) was obtained by alkalinization of a saturated solution of B, the precipitate obtained was rinsed by distilled water until a neutral pH filtrate was obtained. The base was then dried (+40 °C) before its purity was established in comparison with B standard by HPLC. Unless specified otherwise, doses and concentrations of bupivacaine were expressed in term of base equivalent. All other reagents and solvents (Merck, Darmstadt, Germany) were of analytical grade. Freshly prepared distilled water was used as medium throughout the study.

High molecular weight sodium hyaluronate (Na–HA: lot PH271, MW = 1820 kDa, hyaluronic acid content >99%) obtained by gel permeation chromatography was supplied by CAREF (Fougères, France). Sodium hyaluronate was converted to the free hyaluronic acid HA after acidification and dialysis. Briefly, a 1% (w/w) Na–HA gel (pH 5.86) was prepared by hydration of Na–HA fibers in water, then 1 M HCl solution was added to give a final pH of 2. The fluid gel obtained was placed in a molecular-porous membrane tubing (Spectra/Por®, Houston, Texas); molecular weight cut off (MWCO) 12 000–14 000 and was allowed to dialysate 48 h against distilled water (replaced every 12 h) until no more pH variation was seen in the water bath. HA concentration in the gel (1%) was confirmed by gravimetry. BbH ionic complexes as well as BH physical mixtures were formed by addition of appropriate quantities of Bb or B (3 or 6 mg) to 1 ml of the 1% (w/w) HA gel previously obtained or diluted to 0.5% (w/w) by distilled water. All solutions were agitated until complete dissolution of B and 0.5% (w/w) gel intended for in vivo experiments were sterile filtered over a 0.22-μm filter (Minisart® NML, Sartorius, Göttingen, Germany) after the lack of non specific adsorption of bupivacaine to the filter membrane has been checked. Apparent viscosity values measured at 25 °C were 1 cp for B 0.3% solution, 40.34 ± 0.29 cp and 63.66 ± 0.15 cp for 0.5% (w/w) HA gels consisting of BH physical mixture and BbH ionic complex (0.5% w/w gels), respectively. For 1% (w/w) HA gels used for in vitro release experiments, the much higher viscosity values obtained, i.e. between 280 and 300 cp, could not allow their administration through an epidural needle or catheter. The pH was different among the three solutions, i.e. 2.82, 5.85 and 5.65 for BH, BbH and B, respectively.

2.3. In vitro release of bupivacaine from formulations containing hyaluronic acid

The release of bupivacaine from the HA formulations was assessed with a modified USP XX dissolution apparatus thermostated at 37 °C to which was added a polypropylene basket as donor compartment, floating at the surface of the cylindrical spherical bottomed glass flask acting as receptor compartment. Solutions or gels (1 ml) containing 3 mg bupivacaine expressed as equivalent base, were introduced in the donor compartment separated from the acceptor medium (1000 ml distilled water at 37 °C) by a Spectra/por® cellulose membrane; MWCO 12 000–14 000; thickness 35 μm; cross sectional area 3.8 cm^2 (Spectrum, Houston, TX, USA). The membrane MWCO has been chosen to ensure that permeation in the receptor compartment only concerns the free fraction of the drug, i.e. after dissociation or diffusion from the viscous formulations. The sink receptor was stirred with a paddle (100 rpm) and bupivacaine concentration in the receptor was measured continuously at 205 nm and recorded every 15 min during a 6-h period using a UV spectrophotometer (Spectronic1201 Milton, LDC Milton Roy, Riviera Beach, FL, USA).

2.4. Study design

The pharmacokinetics and pharmacodynamics of epidural B, BbH and BH were compared following a cross-over, double-blinded and randomized administration in two groups of rabbits. For the pharmacokinetic analysis study, each animal received an intravenous administration of B (3 or 6 mg) 48 h before the first planned epidural injection. Then, each animal received bupivacaine under the three different formulations, 3 mg in group 1 ($n = 7$), or 6 mg in group 2 ($n = 8$). The time of each injection was 1 min, catheters were flushed after administration with 0.2 ml NaCl 0.9% and a wash-out period of 48 h was allowed between each administration.

2.5. Assessment of effects

Motor block was recorded every minute until maximal intensity was reached, and then every 10 min until complete recovery from spinal anesthesia. The motor block was scored using a four-point scale as follows: level 0 indicated that the rabbit had free movements of hind legs without any limitation; level 1 indicated limited or asymmetrical limb movements for spontaneous body support or walking; level 2 indicated inability to achieve spontaneous support of the back of the body on hind legs; and level 3 indicated a total limb paralysis. Sensory block was assessed by application of painful stimulation with surgical clamp on the posterior legs of rabbits. Complete sensory block was recorded when no motor effect or no increase (or increase less than 10% from baseline value) in heart rate or arterial pressure were observed after painful testing. Time to maximum effect was defined as the time elapsed from the end of epidural injection (T_0) until the maximal score of the block was reached. Total duration time was comprised between the end of injection and complete recovery from the block. Mean arterial blood pressure was continuously monitored from a femoral artery catheter (Sirecust 401.1, Siemens, Erlangen, Germany), the baseline value was recorded after a steady state period of 15 min after arrival of unsedated animals in the operative theatre. After epidural injection, an hypotensive episode was defined as a drop in pressure to less than 30% from the baseline value, and was treated by continuous i.v. administration of dopamine at an initial rate of $10\,\mu g\,kg^{-1}\,min^{-1}$. When blood pressure returned to baseline value and remained stable for at least 5 min, the rate of administration was decreased by $2\,\mu g\,kg^{-1}\,min^{-1}$ from its current level (8, 6, 4, 2 and $0\,\mu g\,kg^{-1}\,min^{-1}$). The total dose and duration of dopamine administration were recorded. Time of maximal decrease in blood pressure and maximal hypotension were recorded. Appearance of systemic side effects was recorded if they occurred (agitation, convulsions) and cardiac rhythm abnormalities were sought by electrocardiographic monitoring through the experiments.

2.6. Drug sampling and analysis

Blood samples (1 ml) were drawn from a catheter placed in a marginal vein of the ear before the injection and then at 0.5, 1.5, 2.5, 3.5, 5, 7.5,10, 15, 30, 45, 60, 120, 180, 240 min. After centrifugation, plasma was collected in polypropylene tubes and stored frozen at −20 °C until analysis. Bupivacaine plasma concentrations were determined after extraction by a reversed phase liquid chromatographic method with UV detection according to Le Guevello et al. (1993) and for which accuracy, within-day and between-day reproducibilities ($n = 10$) at a plasma concentration of $50\,ng\,ml^{-1}$ were 2.7, 3.9 and 4.7%, respectively.

2.7. Pharmacokinetic analysis

All pharmacokinetic parameters, i.e. the first-order absorption rate constant (K_a), the first-order elimination rate constant (K_{el}), the area under the plasma concentration–time curve (AUC), the mean residence time at the site of administration (MRT), the maximum plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), the elimination half-life ($T_{1/2\beta}$) and the total body clearance (CL) were estimated using compartmental analysis. Individual plasma bupivacaine concentration–time profiles obtained after i.v. and epidural administrations were analyzed according to an open-system model with first-order elimination from the central compartment. A bi-compartment model was fitted to the data using a least-squares non linear regression analysis with the Kinetica software package (Innaphase, Philadelphia, USA). The choice of the best weighting scheme and model was based on inspection of standardized weighted residuals versus time plots, and on statistical evaluation of the weighted sum of squared residuals (Boxenbaum et al., 1974). The absolute bioavailability F of bupivacaine following epidural administration of each formulation was determined by the following equation:

$$F = \left[\frac{AUC_{epi}}{AUC_{i.v.}}\right] \times \left[\frac{dose_{i.v.}}{dose_{epi}}\right] \quad (1)$$

where AUC_{epi} and $AUC_{i.v.}$ are the area under the plasma concentration–time curves following epidural and intravenous administration, respectively.

2.8. Statistics

Time to maximum blockade and total duration time of motor and sensory blocks observed among groups and doses were compared using the Kruskal–Wallis

non-parametric test. Relationship between viscosity of formulations and duration of epidural effects was studied by linear regression. Variation in blood pressure was compared among groups by one-way analysis of variance (ANOVA) for repeated measures followed by Scheffe *F*-test. Maximal hypotension, onset time of hypotension and biopharmaceutic parameters were compared among groups by ANOVA followed by Fisher's least significant difference test. Statistical analysis were performed using the software package Staview, version 4.5 (Abacus, USA). *P*-values <0.05 were considered as significant for all comparisons.

3. Results

3.1. In vitro release

When bupivacaine hydrochloride solution (B) was placed in the donor compartment, 86% of the dose reached the receptor in 240 min. For bupivacaine hydrochloride mixture with 0.5% hyaluronic acid (BH formulation), bupivacaine release was slowered due to the gel-viscosity effect with only 29% released at 240 min. When neutral bupivacaine (base form or Bb) was associated to 0.5% hyaluronic acid (BbH formulation), its transfer into the receptor compartment was furthermore decreased (8.4% released at 240 min) owing to complexation between both species, compared to B ionized form for which only viscosity effect occurs (Fig. 1). The rank order release rate of bupivacaine into the aqueous receptor medium was always solution $\gg$ viscous BH formulation $>$ viscous BbH ionic complex. Furthermore, Fig. 1 shows that an increase in formulation viscosity when hyaluronic acid concentration was raised from 0.5 to 1% resulted in a 30% decrease in the amount of bupivacaine released at 240 min.

3.2. In vivo administration: pharmacodynamic evaluation

Transient dysrythmia occurred in 50% rabbits following intravenous administration of 6 mg bupivacaine. No writhing or squeaking was recorded after epidural injections. Two days after the last planned injection, post-mortem examination confirmed the epidural position of the catheter in all animals.

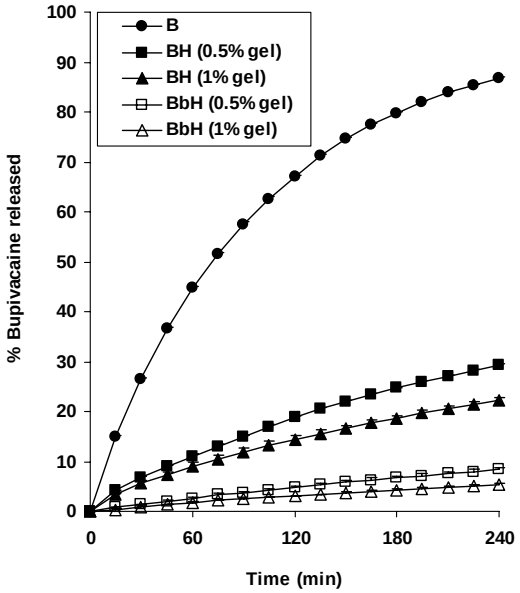

Fig. 1. Percentage bupivacaine released as a function of time from the different formulations containing 3 mg bupivacaine. Each data presented is the mean ± S.D. ($n = 3$ determinations). (●): B; (■): BH (0.5% gel); (▲): BH (1% gel); (□): BbH (0.5% gel); (△): BbH (1% gel). B corresponds to bupivacaine hydrochloride solution, BH corresponds to B physical mixture with hyaluronic acid and BbH to bupivacaine base complexed with hyaluronic acid.

Epidural anesthesia with a 3 mg dose of bupivacaine had similar clinical characteristics whatever the formulation administered. Time to maximum blockade and duration time of motor blocks were not different among groups (Table 1) but especially, when comparing to bupivacaine hydrochloride group, no significant prolongation in total duration of motor effects was found with hyaluronic formulations (115 ± 35% and 133 ± 49% with BH and BbH formulations, respectively, NS). No animal in bupivacaine hydrochloride group had a complete sensory block, it could only be observed in 50% rabbits receiving hyaluronic formulations, i.e. BH and BbH.

In contrast, epidural anesthesia was prolonged with formulations containing hyaluronic acid when bupivacaine was administered at the dose of 6 mg (Table 1). Despite similar time to maximum blockade obtained whether hyaluronic acid was added or not to bupivacaine, we observed prolonged duration of complete motor blocks (149 ± 34% and 142 ± 41% with BH and BbH formulations, respectively, $P <$

Table 1
Time to maximum blockade (TMB) and duration time of motor and sensory blocks in rabbits receiving epidural bupivacaine

	Motor block			Sensory block		
	n	TMB (min)	Duration (min)	*n*	TMB (min)	Duration (min)
3 mg						
BH	7/7	5.0 ± 2.0	113 ± 66	3/7	16.0 ± 4.0	75 ± 20
BbH	6/7	3.0 ± 1.0	97 ± 28	3/7	5.0 ± 2.0	75 ± 35
B	6/7	3.5 ± 1.0	79 ± 23	–	–	–
6 mg						
BH	8/8	4.0 ± 3.0	176 ± 38*	6/8	7.0 ± 4.5	119 ± 36
BbH	6/8	2.0 ± 0.5	165 ± 60*	5/8	2.5 ± 1.0	122 ± 42
B	8/8	2.0 ± 1.5	116 ± 23 *†	8/8	4.0 ± 1.5	73 ± 22††

B corresponds to bupivacaine hydrochloride solution, BH corresponds to B physical mixture with hyaluronic acid and BbH to bupivacaine base complexed with hyaluronic acid.
Data are presented as mean ± S.D.
*$P < 0.02$: 3 mg vs. 6 mg; †$P < 0.02$, ††$P < 0.01$: B vs. BH or BbH.

0.02) and complete sensory blocks (160 ± 70% and 177 ± 72% with BH and BbH formulations, respectively, $P < 0.01$) with hyaluronic formulations. Linear relationships between viscosity of tested solutions and duration of motor blocks ($y = 108 + 0.525x$, $r = 0.274$, $P < 0.02$) as well as complete sensory effects ($y = 75 + 0.508x$, $r = 0.354$, $P < 0.001$) were found (Fig. 2).

Variation of blood pressure was not different among groups excepted the hydrochloride solution (B) that gave more important hypotension after 6 mg than after 3 mg ($P < 0.02$), compared to BH and BbH formulations (Fig. 3).

3.3. *In vivo administration: pharmacokinetics*

Pharmacokinetics parameters of intravenous as well as epidural bupivacaine are displayed in Table 2. Plasma concentration–time profiles of bupivacaine after i.v. (3 mg) and epidural (3 and 6 mg) administrations are shown in Figs. 4 and 5, respectively. In most rabbits, C_{max} following epidural administration occurred at the first sampling time (30 s), showing the rapid absorption of bupivacaine from the epidural space in this model. The C_{max} values were not statistically different among the formulations containing the same amount bupivacaine, while T_{max} were found significantly shorter for B solution compared to BH and BbH formulations, but only in animals receiving 3 mg bupivacaine. Because of the lack of visible absorption phase in some profiles, we could not evaluate individual absorption kinetics using Loo–Riegelman absorption analysis, instead a less accurate estimation of the apparent first-order absorption rate constant K_a was obtained by compartmental modeling of plasmatic data. Result of ANOVA showed that pharmacokinetic

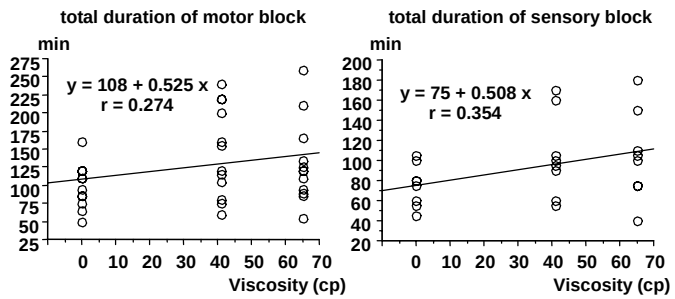

Fig. 2. Relationships between prolongation of both motor and sensory blocks and the viscosity of each bupivacaine containing 6 mg formulation administered epidurally, i.e. bupivacaine hydrochloride solution (viscosity = 1 cp); bupivacaine physical mixture with hyaluronic acid (viscosity = 40 cp) and bupivacaine base complexed with hyaluronic acid (viscosity = 63 cp).

Table 2
Pharmacokinetic parameters after i.v. (3 mg) and epidural (3 or 6 mg) administrations of each formulation tested

	i.v. (3 mg)	B (3 mg)	BH (3 mg)	BbH (3 mg)	B (6 mg)	BH (6 mg)	BbH (6 mg)	Group effect (ANOVA)
K_a (min^{-1})	–	1.92 ± 0.87†*	0.19 ± 0.13*	0.44 ± 0.28*	4.22 ± 1.89	1.07 ± 1.0	0.80 ± 0.57	$P = 0.002$
K_{el} (min^{-1})	0.036 ± 0.026	0.020 ± 0.006	0.024 ± 0.077	0.017 ± 0.048	0.022 ± 0.007	0.044 ± 0.0248	0.028 ± 0.023	$P = 0.168$
AUC (ng min ml^{-1})	30334 ± 11319	27662 ± 4786	30622 ± 7526	33612 ± 9966	81369 ± 4739	72959 ± 3865	77419 ± 3949	$P = 0.0001$
MRT (min)	107.1 ± 22.0	127 ± 22	99 ± 37	123 ± 35	94 ± 25	114 ± 46	108 ± 33	$P = 0.6061$
C_{max} (ng ml^{-1})	1932 ± 1003	944 ± 834†	504 ± 156	915 ± 557	2115 ± 217	3205 ± 1287	1931 ± 558	$P = 0.0001$
T_{max} (min)	–	1.1 ± 0.8†	7.2 ± 6.2*	6.9 ± 5.1*	0.9 ± 0.5	1.01 ± 0.7	1.01 ± 0.9	$P = 0.001$
$T_{1/2\beta}$(min)	89.5 ± 18.4	104.6 ± 25.5	84.5 ± 34.7	116.3 ± 53.5	89.0 ± 33.6	83.9 ± 34.5	82.3 ± 28.1	$P = 0.837$
CL (ml min^{-1})	0.106 ± 0.042	0.095 ± 0.047	0.096 ± 0.023	0.061 ± 0.034	0.057 ± 0.019	0.047 ± 0.033	0.061 ± 0.025	$P = 0.349$
F	–	0.91*	1.01	1.11	1.34	1.2	1.28	$P = 0.001$

B corresponds to bupivacaine hydrochloride solution, BH corresponds to B physical mixture with hyaluronic acid and BbH to bupivacaine base complexed with hyaluronic acid.
Data are presented as mean ± S.D. (i.v.: $n = 15$; group 3 mg: $n = 7$; group 6 mg: $n = 8$).
*$P < 0.05$: 3 mg vs. 6 mg; †$P < 0.05$: B vs. BH or BbH.

parameters, MRT, $T_{1/2\beta}$, CL and K_{el} were not different among the formulations tested (P varying from 0.168 to 0.837). Values of AUC and F were higher when 6 mg of bupivacaine were injected compared to 3 mg, but no difference could be seen between formulations having the same amount bupivacaine. For each dose tested, K_a values were always in the rank order, B ≫ BH > BbH, and despite the lack of accurate estimation due to rapid uptake, the apparent rate of drug uptake into the bloodstream was about four- to six-fold slower after administration of BH or BbH formulations compared to B solution.

4. Discussion

In the present study, our animal model provided accurate data to study epidural anesthesia. All rabbits receiving 3 or 6 mg bupivacaine experienced sensory and motor blocks of increasing durations when the dose of local anesthetic was increased, but prolonged durations of spinal anesthesia with hyaluronic acid were only observed when higher doses of bupivacaine (6 mg) were administered. This has already been reported with the use of slow-release delivery systems. All doses of bupivacaine induced anesthesia, but it seems that only the amounts released from the 6 mg bupivacaine dose delivery systems were higher than those cleared from nerve cells. In contrast, amounts released when only 3 mg of bupivacaine were injected were probably too weak to maintain conduction block at spinal nerves level (Malinovsky et al., 1995).

Previous attempts in order to improve duration of epidural lidocaine were done with hyaluronic acid or lipids. Authors reported a 150–200% prolongation of motor blockade in rabbits with iophendylate (Langerman et al., 1991) and hyaluronic acid (Doherty et al., 1995). In contrast, Doherty et al. (1996) did not find any prolongation of epidural anesthesia with the same formulation of lidocaine in dogs. So, comparisons among different studies and models are difficult to do, because different doses and concentrations of lidocaine were used and mostly only one dose of a new galenic formulation was compared to plain bupivacaine (Doherty et al., 1996). These reasons explain the discrepancies observed among data published. Our study was the first to test epidural hyaluronic formulations of bupivacaine in rabbits. However, in

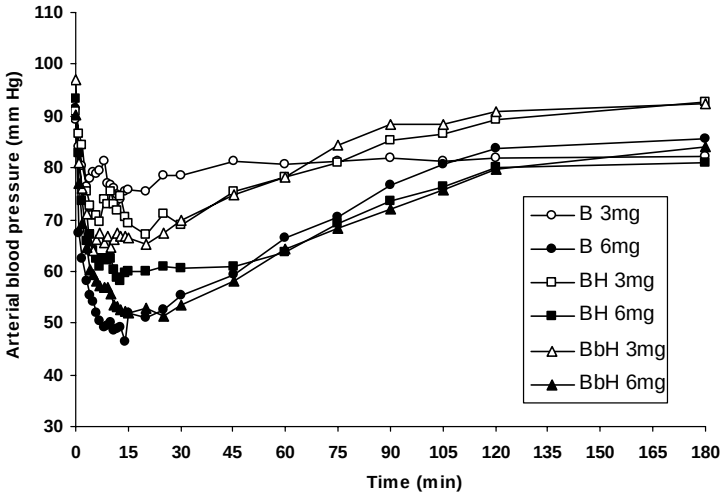

Fig. 3. Variation of mean arterial blood pressure following epidural administration of bupivacaine from different formulations containing 3 mg ($n = 7$) or 6 mg ($n = 8$) bupivacaine. B corresponds to bupivacaine hydrochloride solution, BH corresponds to B physical mixture with hyaluronic acid and BbH to bupivacaine base complexed with hyaluronic acid.

respect to equipotent doses, prolongation observed with mixtures containing hyaluronic acid and bupivacaine was inferior to that observed with lidocaine in rabbits (Doherty et al., 1995). Nevertheless, bupivacaine is a long-lasting local anesthetic, so it is not surprising to obtain a smaller difference in duration of action (50%), compared to the two-fold prolongation of effect obtained with viscous formulation of short-lasting lidocaine (Doherty et al., 1995). Others slow delivery systems of bupivacaine have been previously tested. With the hyaluronic form of bupivacaine we observed a shorter prolongation of anesthesia than that observed by using our microparticular systems, i.e. microspheres and liposomes (Malinovsky et al., 1995, 1999). However, hyaluronic acid is a natural non-immunogenic compound, which has a theoretical advantage over others formulations of local anesthetics. For example, iophendylate used as a lipid carrier is known to induce arachnoiditidis and dextrans are known to induce anaphylactic reactions. So, as

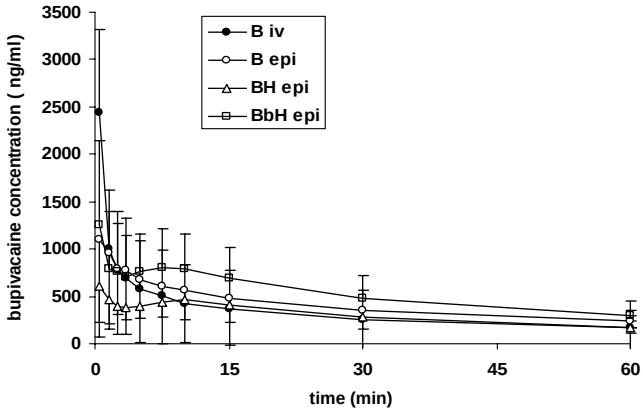

Fig. 4. Plasma concentration–time profiles of bupivacaine after i.v. administration (3 mg) and epidural administration (3 mg) of each formulation tested. Each point is the mean ± S.D. (i.v.: $n = 15$; epidural: $n = 7$). B corresponds to bupivacaine hydrochloride solution, BH corresponds to B physical mixture with hyaluronic acid and BbH to bupivacaine base complexed with hyaluronic acid.

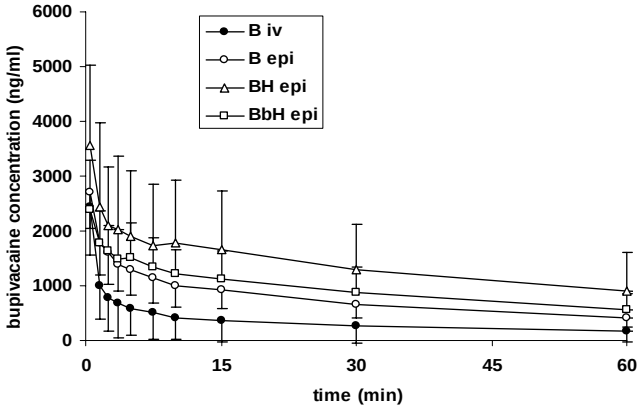

Fig. 5. Plasma concentration–time profiles of bupivacaine after i.v. administration (3 mg) and epidural administration (6 mg) of each formulation tested. Each point is the mean ± S.D. (i.v.: $n = 15$; epidural: $n = 8$). B corresponds to bupivacaine hydrochloride solution, BH corresponds to B physical mixture with hyaluronic acid and BbH to bupivacaine base complexed with hyaluronic acid.

hyaluronic acid is ubiquitous in organism, and do not contain excipient with potential neuroxicity, it may be potentially useful and is readily injectable for spinal administration.

Complexation or mixing of hyaluronic acid with bupivacaine base or hydrochloride, respectively is resulting in higher relative viscosities of such formulations. This is a limiting factor for injection through 23-G diameter needle and catheters commonly inserted in epidural spaces of rabbits. For that reason, we could only use 0.5% hyaluronic acid formulations. We found significant relationships between viscosity of the different formulations injected and prolongation of epidural anesthesia (Fig. 2). Diffusion through viscous polymer may not be the only mechanism for slow release of bupivacaine from such systems, a contribution of electrostatic or ionic mechanisms as been reported to control the release kinetics when cationic bupivacaine is ionically linked to the anionic carboxylate groups of the repeating glucuronic acid residues of hyaluronic acid. In the present study, the pH of the physical mixture (bupivacaine hydrochloride with hyaluronic acid, pH 2.82) was not adjusted to the pH of the ionic complex (bupivacaine base with hyaluronic acid, pH 5.85). In fact, adjusting both viscous formulations to pH of the complex form as previously reported (Doherty et al., 1995) does not allow a comparison of both release mechanisms, as a proportion of bupivacaine hydrochloride in the physical mixture will turn into base form. Nevertheless, we could not differentiate both mechanisms, all happened as if viscosity was strong enough to mask the electrostatic feature of the ionic complex, i.e. viscosity itself controlled the release and prolonged the duration of action.

Among the biopharmaceutics parameters C_{max}, T_{max} and AUC, only T_{max} was modified after epidural administration of BH and BbH compared to plain solution of bupivacaine, and only when a low dose (3 mg) was administered. The lack of difference between AUC following epidural administration of bupivacaine containing viscous formulations showed that the extent of drug release was total whatever the dose administered (3 or 6 mg). It also appeared that pharmacokinetic parameters such as MRT, $T_{1/2\beta}$, CL and K_{el} were not different among the different formulations of bupivacaine used. This seemed due to the lack of formulation effect on drug elimination in our rabbit model, contrasting with the flip-flop pharmacokinetics described by Doherty et al. (1996) for dogs where the apparent terminal elimination phase was in fact absorption. However, the similar reduction in the absorption rate of bupivacaine into the systemic circulation (lower K_a values) for both viscous formulations (ionic complex and physical mixture) compared to the hydrochloride solution (B), whatever the dose (3 or 6 mg) administered, was probably due to a greater fraction of drug reaching the nerve targets. The lower K_a values obtained resulted in a prolonged duration of action, only significantly in the

group receiving 6 mg of bupivacaine with a 45% increase in motor block and a 65% increase in sensory block. Previous studies with viscous formulations of local anesthetics were done in experimental models. Hassan et al. (1985a,b) reported that dextrans, cyclodextrins or hyaluronic acid resulted in viscous formulations thus prolonging infraorbital nerve blocks in rats. They found that no simple correlation other than the relative viscosity could be established. Moreover, this relationship was linear for the lowest viscosity, and a plateau effect was likely with highest viscosity.

In summary, our results suggest that such biocompatible delivery system can allow a slower systemic uptake of bupivacaine avoiding high peak levels and improving the duration of action after epidural administration in the rabbit. The increase in duration of action, even if moderate is similar to what was observed with bupivacaine–cyclodextrin complex, another formulation where the drug is molecularly dispersed (Freville et al., 1996) but smaller than with microparticulate systems such as microspheres (Malinovsky et al., 1995) or liposomes (Malinovsky et al., 1999).

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