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In vitro and in vivo microdialysis calibration using retrodialysis for the study of the cerebrospinal distribution of bupivacaine

Rozenn Clément [a,*], Jean-Marc Malinovsky [b], Gilles Dollo [a], Pascal Le Corre [a], François Chevanne [a], Roger Le Verge [a]

[a] *Laboratoire de Pharmacie Galénique, Biopharmacie et Pharmacie Clinique, Faculté des Sciences Pharmaceutiques et Biologiques, 35043 Rennes Cedex, France*

[b] *Département d'Anesthésie, Hôtel Dieu, Centre Hospitalier Régional Universitaire, 44000 Nantes, France*

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Abstract

Microdialysis coupled to HPLC was used to study the disposition of local anesthetics in the cerebrospinal fluid (CSF) because of the difficulty in sampling CSF. A retrodialysis method for the microdialysis calibration was investigated in vitro and in vivo. Calibration by retrodialysis was simultaneously validated through the use of the zero net flux method. Two local anesthetics (bupivacaine and ropivacaine), which differ structurally by only one methyl group, were respectively utilized as substance of interest and as internal standard. Different parameters were tested in vitro to compare the relative recovery (*RR*) of bupivacaine and the relative loss (*RL*) of ropivacaine. Several flow rates were tried to select an optimal in vivo flow rate (1 μl/min). The *RR* and *RL* values were not influenced by the variation of bupivacaine concentration. A significant variability among different probes within a batch was established (*RR* ranging from 41.1–65.3%; *RL* ranging from 30.7–61.0%). The *K*-factor values, defined as $RL_{\text{ropivacaine}}/RL_{\text{bupivacaine}}$, were calculated in vitro and in vivo. This ratio decreased in vivo but was constant ($K_{\text{in vitro}} = 1.06 \pm 0.04$, $K_{\text{in vivo}} = 0.87 \pm 0.03$). The extracellular tissue concentration of the compound of interest was given by $C = C_{\text{in dialysate}} \times (K/RL)$. Following in vivo implantation in rabbit CSF during 4 h, the probes were tested again in vitro and no deterioration of probe during the in vivo experiment was found. After administration of bupivacaine in the epidural space of rabbits, plasma and microdialysis CSF samples were simultaneously collected. Plasma and CSF disposition of bupivacaine displayed different kinetics. The maximum CSF concentration of B averaged 394 ± 170 μg ml^{-1}, with a mean $T_{\max}$ of 3.8 ± 1.8 min. The maximum plasma concentration of B averaged 0.44 ± 0.09 μg ml^{-1} with a mean $T_{\max}$ occurring at 1 min. Microdialysis, combined with accurate calibration, should be a reliable technique to gain further insight in the spinal disposition of local anesthetics.

Keywords: Microdialysis; Bupivacaine; Ropivacaine; Cerebrospinal fluid; Retrodialysis

* Corresponding author.

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1. Introduction

Local anesthetics are used via epidural routes for major pain control and for regional anesthesia. To reach the target neural tissue of the spinal cord, the drug has to cross the dura and arachnoid mater, to distribute in the cerebrospinal fluid (CSF), and then to cross the pia mater [1]. Because of the difficulty in serially sampling CSF, in vivo data on distribution transfer through the spinal meninges are scarce. In order to improve understanding of the transmeningal passage mechanisms, it is necessary to determine drug concentrations in CSF after epidural administration.

Microdialysis has been applied in pharmacological studies of endogenous compounds like brain neurotransmitters [2]. Currently, there is a growing interest in using this sampling technique for pharmacokinetic and biopharmaceutic studies of exogenous compounds [3–5]. In the current study, microdialysis was chosen as the sampling technique to obtain samples without disturbing CSF flux. The main difficulty with microdialysis analysis is to obtain a reliable in vivo calibration allowing accurate in vivo concentrations to be established.

In the current study, retrodialysis (*RT*) was applied to calibrate microdialysis probes. This calibration technique is based on the principle that the relative loss (*RL*) of a carefully chosen internal standard, added to the perfusate, is related to the relative recovery (*RR*) of the substance of interest [6]. Bupivacaine and ropivacaine, which differ structurally by only one methyl group, were respectively utilized as substance of interest and as internal standard to study the disposition of bupivacaine after bolus epidural administration. The calibration was simultaneously validated through the use of the zero net flux method where recovery in vivo was estimated from dialysate concentrations in a wide range of concentrations, while maintaining the extracellular concentration at steady state [7]. Due to the toxicity of bupivacaine precluding the obtention of steady state concentration, the calibration zero net flux method was only performed in vitro. The *K*-factor, defined as the ratio between *RL* of internal standard and *RL* of substance of interest, was used to correct the dialysate concentrations and to determine the bupivacaine CSF concentrations. The extracellular concentration of bupivacaine was then calculated according to: $C = C_{\text{dialysate}} \times (K/RL)$ [8].

2. Materials and methods

2.1. Chemicals

Bupivacaine (substance of interest), ropivacaine (internal standard of microdialysis) and etidocaine (external standard of HPLC) were supplied by Astra (Astra Pain Control, Sweden).

The composition of Ringer's solution was NaCl 8.6 g l^{-1}, KCl 0.33 g l^{-1}, $CaCl_2$, 2 H_2O 0.3 g l^{-1}.

All other reagents were of analytical grade.

2.2. Microdialysis in vitro and in vivo

Microdialysis sampling was performed using a CMA/102 microinjection pump coupled to a microdialysis probe CMA/20 (membrane length 10 mm, 0.5 mm outer diameter, molecular weight cut off 20 kDa, CMA Microdialysis, Sweden). Samples were collected every 2 min during a 1-min interval. Dialysate (1 μl) was collected using an automatic sampler in vials containing 100 μl of a Ringer's solution containing etidocaine (1 μg ml^{-1}). An aliquot (50 μl) was immediately injected onto the chromatographic system.

2.2.1. In vitro experiment

(1) The probe was placed in a quiescent solution of bupivacaine at room temperature and was perfused with a solution of ropivacaine. The influence of bupivacaine concentration (10, 20, 50, 100, 200 μg ml^{-1}, six measurements at each concentration) and the influence of flow rate (0.8, 1, 1.2, 1.5, 2 μl min^{-1}) were investigated to compare *RR* of bupivacaine and *RL* of ropivacaine. The *RR* and the *RL* were calculated as follows:

$$RR = \frac{C_{\text{dialysate}}}{C_{\text{extracellular}}} \times 100$$

$$RL = \frac{C_{perfusate} - C_{dialysate}}{C_{perfusate}}$$

where $C_{dialysate}$ is the concentration in dialysate, $C_{extracellular}$ is the concentration in the medium surrounding the probe and $C_{perfusate}$ is the concentration in the perfusate medium.

(2) The probe was placed in a quiescent solution of bupivacaine (10 μg ml^{-1}) at room temperature and was perfused with a solution containing ropivacaine (100 μg ml^{-1}) and varying concentrations of bupivacaine (5, 10, 20, 50, 100, 200 μg ml^{-1}). The *RL* of ropivacaine was determined and compared to the *RR* values of bupivacaine obtained by using the zero net flux method.

(3) The probe was placed in a quiescent Ringer's solution at room temperature and was perfused with a solution containing bupivacaine (100 μg ml^{-1}) and ropivacaine (100 μg ml^{-1}). The *K*-factor, defined as the ratio between *RL* of ropivacaine and *RL* of bupivacaine, was determined ($n = 10$ for each probe tested).

2.2.2. In vivo experiment

Immediately after probe insertion in the subarachnoidal space, an in vivo calibration with determination of *K*-factor ($n = 10$ for each probe tested) was achieved over a period of 30 min.

After in vivo calibration, the probe was perfused throughout the experiment, at 1 μl min^{-1} with a Ringer's solution containing ropivacaine (100 μg ml^{-1}). The lag time between the end of the calibration and the beginning of the experiment was 60 min. During the experiment, *RL* of ropivacaine was determined in each sample.

2.3. Animals

The study was approved by the local Committee of Laboratory Animal Care in accordance with the rules and guidelines concerning the care and use of laboratory animals. New Zealand adult female rabbits ($n = 5$) were housed individually in standard cages. They were provided free access to food and water.

Throughout the experiment, animals were sedated with intermittent intravenous pentobarbital (25 μg ml^{-1}). Following L5–L6 laminectomy, a microdialysis probe was inserted in the lumbar subarachnoidal space (i.e. in contact with CSF) [9]. Then, a guided epidural catheter was inserted into the epidural space, in order to put the tip of the epidural catheter opposite the tip of the probe. This catheter was used to inject bupivacaine. A catheter was placed in the femoral artery for blood sampling.

2.4. Study design

A bupivacaine solution (2 mg in 1 ml) was injected over 30 s into the epidural space of rabbits. CSF dialysates samples were collected over a 60-min period according to the following schedule: every 2 min for 30 min and then every 4 min for the remaining 30 min. Blood samples were drawn over 60 min at 0.5, 1, 3, 5, 7, 11, 23, 31, 47, and 60 min. The blood samples were immediately centrifuged and plasma was stored frozen until analysis. Bupivacaine in plasma was extracted from plasma samples according to a previously published method [10].

2.5. Analysis

The CSF dialysates and the blood samples were analysed by high pressure liquid chromatography with UV absorbance detection ($\lambda = 205$ nm).

The chromatographic system consisted of a Waters Model 6000A pump (Waters, Milford, MA) equipped with a Waters Model WISP 717 automatic injector, an LDC Milton Roy Model Spectromonitor 3100 variable-wavelength detector (LDC Milton Roy, Riviera Beach, FL), and a Delsi Model Enica 21 integrator (Delsi, Suresne, France). The analytical chromatographic column was a Lichrospher RP-B Merck (length 125 mm, internal diameter 4 mm). The flow rate was 1 ml min^{-1}, and the temperature was maintained at 30°C. The mobile phase consisted of acetonitrile (230 ml) and pH 2.1, 0.01 M sodium dihydrogenphosphate (770 ml).

2.6. Data analysis

Data were presented as mean ± S.D. A value of $P < 0.05$ was considered significant. The influence of bupivacaine on *RR* and *RL* was analysed by one-way ANOVA.

3. Results and discussion

The chromatograms in Fig. 1 displayed the selectivity (selectivity factor > 1.2) of the separation of bupivacaine, ropivacaine and etidocaine in CSF dialysate and in plasma samples. The limits of quantification for bupivacaine in CSF dialysate and in plasma samples were 1 μg ml^{-1} and 2 ng ml^{-1}, respectively.

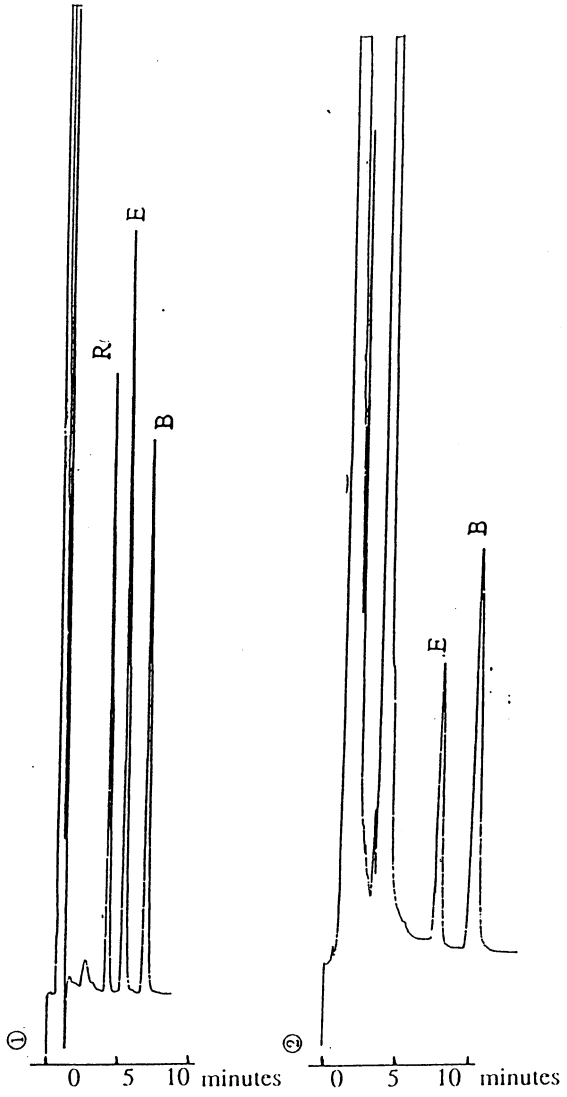

Fig. 1. Chromatograms of CSF dialysate sample containing 100 μg ml^{-1} of B, R, E (1) and of extracted plasma sample containing 100 ng ml^{-1} of B and 50 ng ml^{-1} of E (2). B, bupivacaine; E, etidocaine; R, ropivacaine.

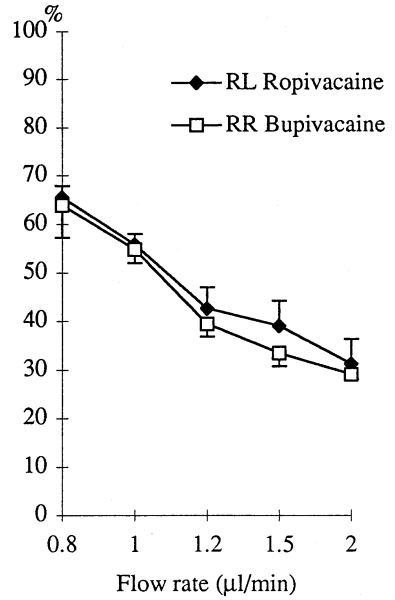

Fig. 2. Effect of flow rate on relative recovery (*RR*) of bupivacaine and on relative loss (*RL*) of ropivacaine. Data were expressed as mean ± S.D. ($n = 6$).

In a first step, the influence of flow rate on *RR* of bupivacaine and on *RL* ropivacaine was studied. As shown in Fig. 2, the increase in flow rate (from 0.8 to 2 μl min^{-1}) led to a twofold decrease in both parameters. Therefore, a flow rate of 1 μl min^{-1} was selected for all the following experiments.

Ungerstedt [11] and Scheller and Kolb [6] have shown respectively that the *RR* and the *RL* are independent of concentration. We have investigated the influence of bupivacaine concentration on both parameters. As described in Table 1, variations of bupivacaine concentration within a range from 10 to 200 μg ml^{-1} influenced neither the *RR* of bupivacaine nor the *RL* of ropivacaine (ANOVA not significant).

It should be stressed that there was a significant variability of both *RR* and *RL* values between probes of the same batch ($n = 10$ probes). The mean value of *RR* (min–max) was 52.8 ± 7.1% (41.1–65.3%). The mean value of *RL* (min–max) was 49.0 ± 8.9% (30.7–61.0%). Such a variability between probes, which had not yet been des-

Table 1
Effect of bupivacaine concentration on relative recovery (*RR*) bupivacaine and on relative loss (*RL*) ropivacaine values

Bupivacaine concentration (μg ml^{-1})	*RL* Ropivacaine (%)	*RR* Bupivacaine (%)
10	55.1 ± 3.2	43.5 ± 4.7
20	53.1 ± 3.9	43.9 ± 0.1
50	54.1 ± 6.7	43.0 ± 5.4
100	53.7 ± 3.5	41.9 ± 2.1
200	53.6 ± 2.1	40.9 ± 2.1

Results are expressed as mean ± S.D. ($n = 6$ samples per concentration tested).

cribed, might result from a variation in the surface of the dialysis membrane. As a result, it seemed necessary to choose preferably retrodialysis as the calibration method.

Fig. 3 displays the results of the zero net flux study, expressed as mean (± S.D.) ($n = 6$ samples for each concentration tested). The regression slope, which was an estimate of *RR*, was 0.56 ± 0.08. Retrodialysis was performed simultaneously and the *RL* determined in each sample was 0.54 ± 0.05. The agreement between the zero net flux recovery and retrodialysis was assessed and suggested the absence of interaction across the probe membrane in opposite directions.

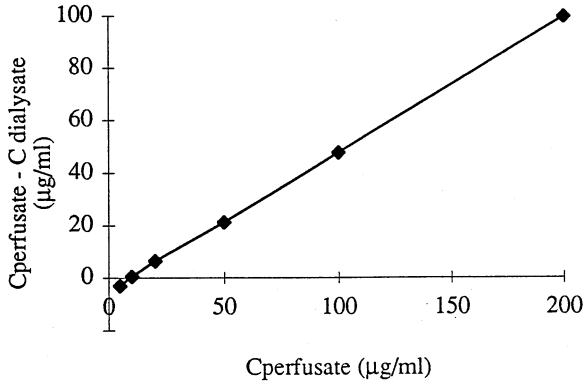

Fig. 3. Illustration of zero net flux method with $C_{perfusate}$ as the concentration of bupivacaine in the perfusion medium and $C_{dialysate}$ as the concentration of bupivacaine in the dialysate ($n = 6$ samples for each concentration tested). The slope is an estimate of the recovery.

The *K*-factor determined in vitro for eight probes ranged from 1.00 to 1.11. For each probe, the variability in *K*-factor was lower than 3.5% ($n = 10$). In vivo, *K*-factor decreased, ranging from 0.82 to 0.93. For each probe ($n = 7$), the coefficient of variation was lower than 5.1% ($n = 10$).

Such a difference between in vitro and in vivo values for *K*-factor may result from a difference in tissue affinity between bupivacaine and ropivacaine as a result of a difference in liposolubility. The partitioning coefficients between *n*-heptane and phosphate buffer of bupivacaine and ropivacaine were respectively 20.5 and 6.1 [12].

The in vitro/in vivo difference in *K*-factor value meant performing an in vivo calibration before the experiment in order to correct the dialysate concentrations to avoid overestimating the tissular concentrations.

Following in vivo implantation in rabbit CSF during 4 h, the probes were tested in vitro and a very slight, although significant, variation in *K*-factor values was found. The *K*-factor values determined before and after in vivo probe insertion were 1.08 ± 0.03 and 1.03 ± 0.01, respectively. This suggested the lack of significant deterioration of the probes during the in vivo experiment.

In this preliminary study achieved in five rabbits, CSF and plasma profiles of bupivacaine have been determined. The mean unbound CSF and total plasma concentration of bupivacaine are presented in Fig. 4. The unbound CSF maximal concentration of bupivacaine was around 900-fold higher than in plasma (394 ± 170 μg ml^{-1} vs. 0.44 ± 0.09 μg ml^{-1}). The time for maximal concentration (T_{max}) was 3.8 ± 1.8 min in CSF and 1.0 min in plasma.

4. Conclusion

The experimental results indicated that ropivacaine can be used as an internal standard for studying CSF bupivacaine kinetics. The microdialysis sampling technique allowed determination of CSF concentration of bupivacaine after epidural administration. This preliminary work

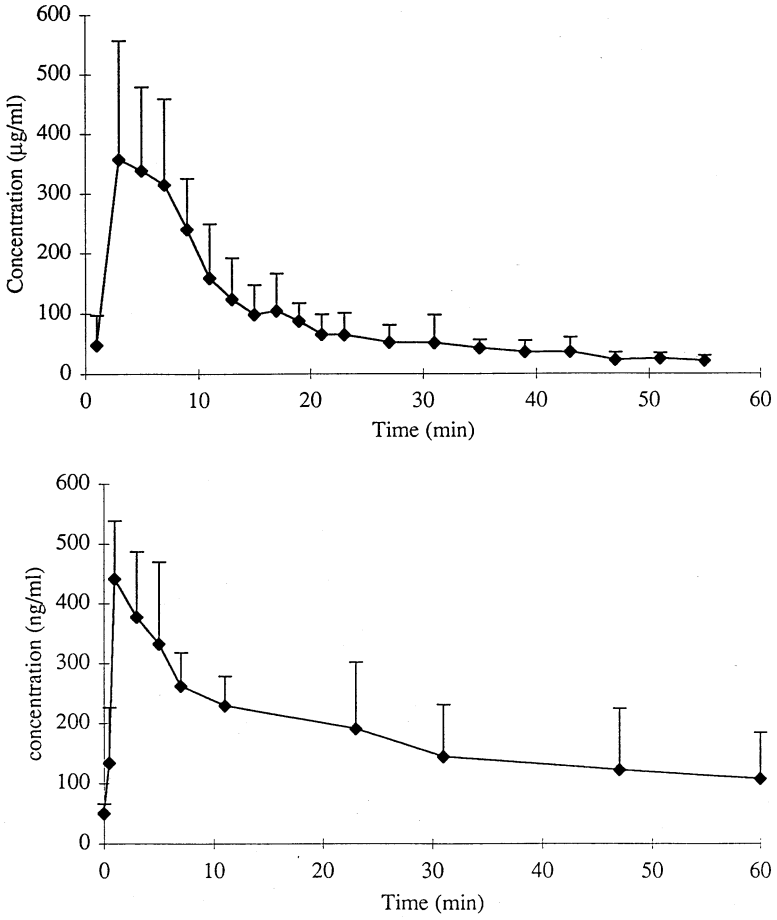

Fig. 4. Mean (±S.D.) CSF concentration–time profile of bupivacaine (top curve, μg ml^{-1}) and mean (±S.D.) plasma concentration–time profile of bupivacaine (bottom curve, ng ml^{-1}) after epidural administration of 2 mg of bupivacaine in five rabbits.

has shown that microdialysis should be a promising tool to gain further insight into epidural disposition of drugs and to improve understanding of transmeningal diffusion mechanisms.

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