

Cerebrospinal Fluid Bioavailability and Pharmacokinetics of Bupivacaine and Lidocaine after Intrathecal and Epidural Administrations in Rabbits Using Microdialysis

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Accepted for publication January 7, 1999 This paper is available online at <http://www.jpvet.org>

ABSTRACT

The aim of this work was to study the cerebrospinal fluid (CSF) bioavailability and pharmacokinetics of bupivacaine (BUP) and lidocaine (LID) administered separately in rabbits using microdialysis with retrodialysis calibration. Microdialysis probe and catheters were inserted under control of the view in the intrathecal or epidural spaces. The epidural disposition of BUP and LID after epidural administration of low (0.69 μM) and high (6.9 μM) doses was studied. Then, the intrathecal and plasma dispositions after separate intrathecal (0.2 μM) and epidural administration (6.9 μM) were investigated. The CSF binding of BUP and LID was linear in a range from 50 to 500 $\mu\text{g/ml}$, and the mean unbound CSF fraction at a concentration of 100

$\mu\text{g/ml}$ was $39.3 \pm 2.3\%$ for BUP and $75.8 \pm 7.7\%$ for LID. Epidural and intrathecal disposition of BUP and LID showed a biexponential decline. After epidural administration, the CSF concentrations of BUP and LID were much higher than those in plasma. After intrathecal administration, the plasma concentrations were below the limit of quantitation. Although the absorption rate of BUP appeared higher than that of LID, the mean CSF bioavailability of epidural BUP and LID was 5.5 and 17.7%, respectively. The unexpectedly higher CSF bioavailability of LID, the less lipophilic drug, may result from the difference in the processes competing for drug epidural removal.

Epidural and intrathecal local anesthetics, opioids, and α_2 -adrenergic agonists (i.e., clonidine) are commonly used during the postoperative period to relieve pain. Opioids and clonidine act by receptor binding at the spinal level and at the systemic level after absorption in the systemic circulation and subsequent brain distribution. In contrast, local anesthetics act by inhibition of nerve influx transmission only at the spinal level, but their systemic absorption after epidural administration is significant and leads to systemic adverse effects such as cardiac and neurologic toxicities.

After epidural administration, these drugs need to cross the spinal meninges (i.e., dura mater and arachnoid mater) to reach their site of action. However, if the spinal disposition of opioids and clonidine has been studied extensively, the

spinal disposition of local anesthetics has been investigated poorly. This has been done only once for local anesthetics (Wilkinson and Lund, 1970), and frequently for opioids (Nordberg et al., 1983; Gourlay et al., 1987; Hansdottir et al., 1991, 1995; Taverne et al., 1992) and clonidine (Eisenach et al., 1993, 1995). The distribution of these agents in cerebrospinal fluid (CSF) has been studied after repeated intrathecal punctures or insertion of indwelling intrathecal catheters to withdraw CSF. These studies demonstrated a rather low CSF bioavailability lower than 4% for pethidine, morphine, and sufentanil and 14% for clonidine. Moreover, a relationship between CSF concentration and analgesic effect has been described for clonidine (Eisenach et al., 1993).

Using experimental *ex vivo* models, authors have studied extensively the permeability of dura mater (Moore et al., 1982; McEllistrem et al., 1993) or meninges (Bernards and

Received for publication July 10, 1998.

ABBREVIATIONS: CSF, cerebrospinal fluid; BUP, bupivacaine; LID, lidocaine; RL, relative loss; RR, relative recovery; C_{max} , maximum total plasma concentration; $C_{\text{max-epi}}$, maximum unbound epidural concentration; T_{max} , peak plasma concentration time; $T_{\text{max-CSF}}$, peak CSF concentration time; AUC_{last} , area under CSF concentration-time curves from the time of drug administration up to the last sampling point; $\text{AUC}_{\text{CSF-it}}$, area under the unbound CSF concentration-time curve up to the last sampling point after intrathecal administration; $\text{AUC}_{\text{CSF-epi}}$, area under the unbound CSF concentration-time curve up to the last sampling point after epidural administration; AUC_{inf} , area under CSF concentration-time curves from the time of drug administration to infinity; K_{10} , elimination rate constant; K_{12} and K_{21} , distribution rate constants; K_a , absorption rate constant; V_1 , initial volume of distribution; V_{ss} , steady-state volume of distribution; F_{CSF} , CSF bioavailability; C_{inj} , drug concentration of the solution injected; CL_E , elimination clearance; CL_i , intercompartmental clearance.

Hill, 1990) to different drugs. They showed that a simple passive diffusion mechanism is likely. Relationships between permeability and different physicochemical properties were studied but the results appeared controversial depending on the models (dura alone or all meninges) and on the nature of meninges (humans or animals). However, these *ex vivo* models do not take into account the gradient of pressure existing between epidural and intrathecal spaces, the meningeal surface area in contact with solution injected epidurally, uptake by epidural venous blood vessels, and uptake into epidural tissues such as epidural fat.

Given the paucity of data on local anesthetics, excepting *ex vivo* studies, *in vivo* investigations thus are strongly required to understand the spinal disposition of these drugs and to study the *in vivo* drug release from drug delivery systems for local anesthetics currently investigated to improve the clinical and toxicological features of these drugs (Boogaerts et al., 1995, Le Corre et al., 1995, Fréville et al., 1996). We recently validated the use of microdialysis coupled to HPLC to study the intrathecal disposition of bupivacaine (BUP) in rabbits (Clément et al., 1998). This technique, allowing measurement of drug concentrations without removing CSF, also permits access to concentrations of local anesthetics in the epidural space, where it is impossible to withdraw any epidural fluid because of cephalad and caudal spread of the solution injected epidurally (Paul et al., 1989). The aim of this study was to investigate the spinal disposition of lidocaine (LID) and bupivacaine after epidural and intrathecal administration in awake rabbits.

Materials and Methods

Chemicals. Local anesthetics (bupivacaine, etidocaine, lidocaine, and ropivacaine) were supplied by Astra (Astra Pain Control, Sweden). A Ringer's solution (8.6 g/liter NaCl, 0.33 g/liter KCl, 0.3/liter CaCl₂, 2H₂O) was used as perfusion fluid. All other reagents were of analytical grade.

Animals. The study was approved by the Committee of Laboratory Investigation and Animal Care of our institution and performed in accordance with French Ministry of Agriculture laws and guidelines for laboratory animal experiments. Experiments were performed on female New Zealand albino rabbits, weighing 3.0 ± 0.3 kg, housed individually in standard cages with free access to food and water. Animals were fasted the night before the experiment.

Animal Preparation. After catheterization of a marginal vein of ear, anesthesia was induced with 25 mg of 2.5% thiopental. Insertion of spinal and vascular catheters was performed under epidural procaine anesthesia and light sedation, maintained with *i.v.* 1% thiopental (5–10 mg). The technique of epidural anesthesia was described elsewhere (Malinovsky et al., 1997). In the supine position, the femoral area was dissected surgically and an arterial 20-gauge catheter was inserted via the femoral artery. The catheter was heparinized and then closed until blood sampling.

Then, with the rabbit in prone position, the back was incised between the spinous process of L5 and L6 vertebrae after soft surgical dissection of the flavum ligamentum. If the area appeared to be not large enough to introduce spinal catheters for local anesthetic administration and microdialysis probe, a small laminectomy was performed. Under control of the view, the catheters were inserted either in epidural or intrathecal space after surgical incision of meninges. Catheters inserted for administration of local anesthetics were flushed previously using 0.9% sodium chloride. When catheters for local anesthetic administration and microdialysis probe were not placed in the same space, they were inserted to put the tip of catheter opposite to the tip of the probe.

Microdialysis Conditions. Microdialysis sampling was performed using a CMA/102 microinjection pump coupled to a microdialysis probe CMA/120 (membrane length, 10 mm; 0.5-mm outer diameter; molecular mass cut-off, 20 kDa; CMA Microdialysis, Sweden). Microdialysis samples were collected every 2 min during a 1-min interval. The *in vitro* study of the kinetics of probe response showed that a lag time of 3 min was necessary to obtain the experimental drug concentration (data not shown). Hence, in all the *in vivo* experiments the first experimental drug concentration considered was that measured at 3 min. Dialysates (sample volume = 1 μl) were collected in vials containing 100 μl of etidocaine (1 μg/ml), and a 50-μl aliquot was injected onto the chromatograph.

Microdialysis Calibration. Retrodialysis, using ropivacaine as internal standard (IS), was applied to calibrate the 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 (SI) (Scheller and Kolb, 1991). The K factor values, defined as the ratio RL_{IS}/RL_{SI}, were used to determine the extracellular concentration of the compounds of interest according to $C = C_{\text{dialysate}} \times K/RL_{\text{IS}}$ (Deleu et al., 1995). The validation of the calibration was assessed by comparing retrodialysis with the zero-net flux method, where the recovery is estimated from dialysate concentrations in a wide range of concentrations while maintaining the extracellular concentration at steady state (Wang et al., 1993).

To validate retrodialysis using ropivacaine as internal standard for lidocaine, the following experiments were performed.

1. The probe, placed in a solution of LID (10 μg/ml), was perfused with a solution containing ropivacaine (100 μg/ml) and varying concentrations of LID (5, 10, 20, 50, 100, and 200 μg/ml). The RL of ropivacaine was determined and compared with the RR values of LID obtained by using the zero-net flux method. Because of the systemic toxicity of LID, precluding the steady-state concentrations to be obtained, the comparison between the two methods was performed *in vitro*. These experiments showed a good agreement between the RL of ropivacaine (RL = 0.44 ± 0.03) and the RR value of LID (RR = 0.48 ± 0.09).

2. The probe, placed in a Ringer's solution, was perfused with a solution containing LID (100 μg/ml) and ropivacaine (100 μg/ml), and the K factor was determined *in vitro*. Immediately after probe insertion in the subarachnoid space of rabbits, an *in vivo* calibration with determination of K factor was achieved over a period of 30 min. The LID K factors determined *in vitro* and *in vivo* were 1.01 ± 0.05 and 1.09 ± 0.08, respectively.

As shown previously (Clément et al., 1998), ropivacaine can be used as an internal standard to study the disposition of BUP (RL ropivacaine = 0.54 ± 0.05, RR BUP estimated by zero-net flux method; RR = 0.56 ± 0.08, K_{in vitro} = 1.06 ± 0.04, K_{in vivo} = 0.87 ± 0.03).

After *in vivo* calibration, the probe was perfused throughout the experiment at a flow rate of 1 μl/min with a Ringer's solution containing ropivacaine (100 μg/ml). 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 and used to correct the dialysate concentrations.

Evaluation of Unbound Fraction of BUP and LID in CSF. CSF samples (between 1.5 and 2 ml) were withdrawn from the cisterna magna by puncture through the atlanto-occipital membrane in six rabbits. Individual CSF samples from three rabbits were divided in two aliquots for the separate evaluation of BUP and LID binding at a concentration of 100 μg/ml. CSF samples from three rabbits were pooled and then divided in two aliquots for the separate evaluation of BUP and LID binding at the following concentrations: 50, 100, 250, and 500 μg/ml. The binding was studied using microdialysis by spiking a CSF aliquot either with BUP or LID.

Chromatographic Analysis of BUP and LID. The separation and quantification of the local anesthetics in the dialysate (CSF, epidural samples) or in plasma samples were carried out using a

HPLC method with UV absorbance detection ($\lambda = 205$ nm). Dialysate samples were injected immediately onto the chromatographic system. The blood samples were centrifuged and plasma was stored frozen until analysis. BUP and LID in plasma were extracted from plasma before analysis by HPLC according to a previously published method (Le Guevello et al., 1993). The limit of quantification of BUP and LID was 3 $\mu\text{g/ml}$ and 1.5 $\mu\text{g/ml}$ in dialysate and 4 ng/ml and 2 ng/ml in plasma, respectively.

The chromatographic system consisted of a Waters model 6000A pump (Waters, Milford, MA) equipped with a Waters model 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, 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 0.01 M sodium dihydrogenphosphate, pH 2.1.

Study Design. CSF disposition of local anesthetics only has been studied in each animal either after intrathecal administration or after epidural administration because meningeal puncture with a needle diameter size larger than 0.4 mm (equivalent to the hole made with the 24-gauge needle) significantly increases the flux of local anesthetics through meninges (Bernards et al., 1994).

In a first part, we investigated in three animals the epidural disposition of BUP and LID after separate epidural administration. Equimolar low doses (0.69 μM , 0.200 mg of BUP, and 0.162 mg of LID) and high doses (6.9 μM , 2.00 mg of BUP, and 1.62 mg of LID) were administered in 30 s under a volume of 1 ml. A series of nine concentration-time profiles was obtained for both BUP and LID. Epidural sampling was achieved every 2 min over 45 min.

In a second part, we investigated in six animals the intrathecal disposition of BUP and LID after separate intrathecal administration. Equimolar doses (0.2 μM , 0.060 mg of BUP, and 0.049 mg of LID) were administered in 30 s under a volume of 0.1 ml. A series of six and four concentration-time profiles was obtained for BUP and LID, respectively. Intrathecal sampling was achieved every 2 min over the 30 first min and then every 4 min over the following 24 min. Blood sampling (1.5 ml) was achieved in each animal according to the following schedule: before administration and then at 0.5, 1, 3, 5, 7, 11, and 15 min.

In a third part, we investigated in nine animals the intrathecal and plasma disposition of BUP and LID after separate epidural administration. Equimolar doses (6.9 μM , 2 mg of BUP, and 1.62 mg of LID) were administered in 30 s under a volume of 1 ml. A series of seven intrathecal concentration-time profiles and five plasma concentration-time profiles was obtained for BUP and LID, respectively. Intrathecal sampling was achieved every 2 min over the 30 first min and then every 4 min over the following 24 min. Blood sampling (1.5 ml) was achieved in each animal according to the following schedule: before administration and then at 0.5, 1, 3, 5, 7, 9, 21, 29, 45, and 55 min.

Pharmacokinetic Analysis. Intrathecal- and epidural-population pharmacokinetic parameters of BUP and LID were determined using the statistical pharmacokinetic software P-Pharm (version 1.5; Innaphase, Champs sur Marne, France). Intrathecal concentration data after intrathecal administration and epidural concentration data after epidural administration were fitted according to a biexponential model. Intrathecal concentration data after epidural administration were fitted according to a triexponential model. The distribution of the random effect was assumed as normal, and the residual error variance was assumed as heteroscedastic (in proportion to the squared value of the predictions). Initial population parameter estimates were derived from the mean of the individual parameter values obtained by using a stripping algorithm. Individual parameters for each data set (Bayesian estimates) were obtained from the current population parameters and the individual data.

The maximum total plasma concentration, the maximum free epidural and intrathecal concentration (C_{max}), and the correspond-

ing time (T_{max}) were derived from raw data. Areas under CSF concentration-time curves from the time of drug administration up to the last sampling point (AUC_{last}) were computed by the linear trapezoidal rule by using a noncompartmental model with the software package WinNonlin (version 1.1; Scientific Consulting Inc., Apex, NC). Because both epidural and intrathecal administrations were not performed in the same animals, a mean CSF bioavailability (F_{csf}) was determined by the following: $F_{\text{csf}} = (\text{mean AUC}_{\text{csf-epi}}/\text{epidural dose})/(\text{mean AUC}_{\text{csf-it}}/\text{intrathecal dose})$.

Statistics. All data are presented as mean \pm S.D. Student's *t* test was used to compare individual means. A *P* value less than .05 was considered as statistically significant.

Results

Epidural Microdialysis after Epidural Administration. The individual epidural concentration-time profiles of BUP and LID after equimolar epidural administration (low and high doses) are presented in Fig. 1. The values of pharmacokinetic parameters after the administration of the high doses of BUP and LID are listed in Table 1. Pharmacokinetic modeling was achieved only after the administration of the high dose (i.e., the dose used in the intrathecal evaluation after epidural administration) because too few data were obtained in the terminal elimination phase after the administration of the low dose. The epidural concentration-time curves showed a biphasic decline with an apparent terminal elimination phase occurring earlier for BUP.

The high-dose to low-dose ratio of maximum unbound epidural concentration ($C_{\text{max-epi}}$) averaged 13 and 11 for BUP and LID, respectively. The ratio between $C_{\text{max-epi}}$ and the corresponding concentration of the injected solution (C_{inj}) was $26.5 \pm 10.3\%$ and $35.3 \pm 14.5\%$ ($P = .32$) after the administration of low and high doses of BUP, respectively. After the administration of low and high doses of LID, this ratio averaged $54.1 \pm 25.2\%$ and $60.5 \pm 17.3\%$ ($P = .70$), respectively. These data showed that the ratio was not dependent on the injected dose but was dependent on the drug injected ($P = .007$). During the terminal apparent elimination phase, the level of epidural concentration of BUP and LID after the high dose was about 10 times higher than those obtained after the low dose in accordance with the difference in dosing.

Intrathecal Microdialysis after Intrathecal Administration. Individual CSF concentrations of BUP and LID after equimolar intrathecal administration are illustrated by Fig. 2 and show a biexponential decline. The pharmacokinetic parameters are presented in Table 2. The concentrations of BUP and LID in plasma were beyond the limits of detection during the intrathecal experiment. The $\text{AUC}_{\text{last}}/\text{area under CSF concentration-time curves from the time of drug administration to infinity}$ (AUC_{inf}) ratio of intrathecal BUP (87.8%) and LID (91.7%) indicated that the sampling period was long enough to obtain a suitable description of the CSF disposition of these drugs.

Intrathecal and Plasma Disposition after Epidural Administration. Figure 3 shows the individual CSF concentrations of BUP and LID after administration of an epidural equimolar dose. The CSF pharmacokinetic parameters are presented in Table 3. The $\text{AUC}_{\text{last}}/\text{AUC}_{\text{inf}}$ ratio of intrathecal BUP (85.5%) and LID (95.3%) indicated that the sampling period was long enough to obtain a suitable description of the

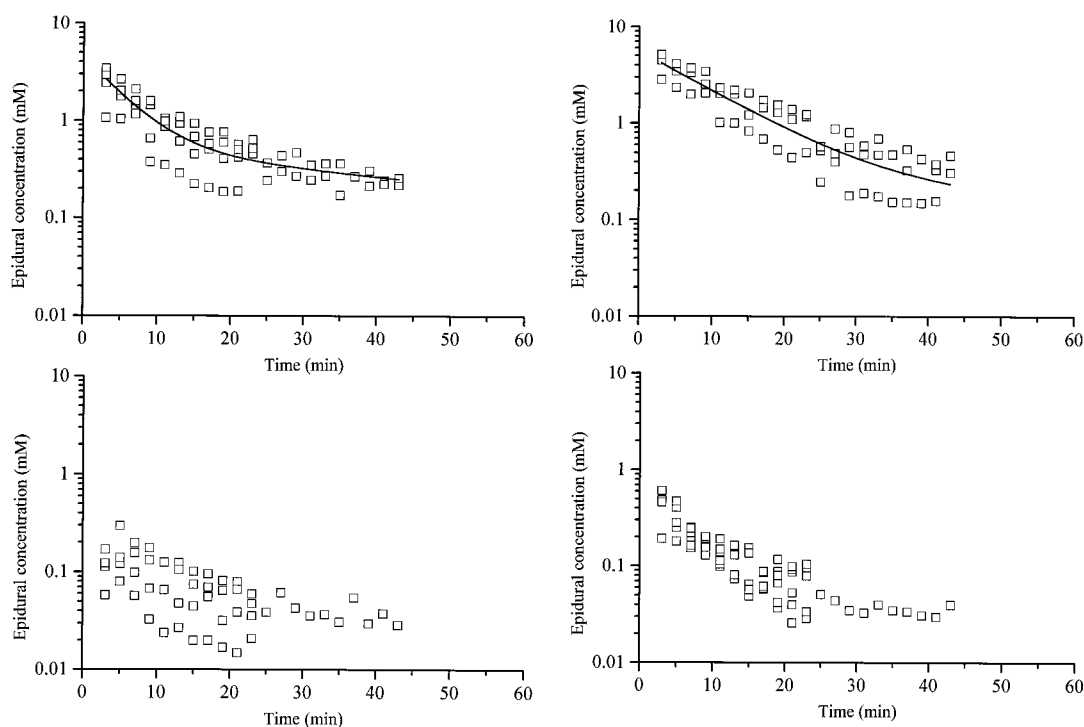


Fig. 1. Measured and population-predicted epidural concentrations of bupivacaine (left) and lidocaine (right) after separate epidural administration of equimolar low dose (bottom, $0.69 \mu\text{M}$: 0.2 mg of BUP and .162 mg of LID) and high dose (top, $6.9 \mu\text{M}$: 2 mg of BUP and 1.62 mg of LID) in rabbits.

TABLE 1

Epidural pharmacokinetic parameters (mean \pm S.D.) of BUP and LID after separate epidural administration of equimolar dose ($6.9 \mu\text{M}$: 2 mg of BUP and 1.62 mg of LID) in rabbits

	BUP	LID
C_{max} ($\mu\text{g/ml}$)	705 ± 289	980 ± 280
(mM)	2.45 ± 1.00	4.15 ± 1.18
CL_E (ml/min)	0.140 ± 0.011	$0.084 \pm 0.032^*$
CL_I (ml/min)	0.142 ± 0.074	0.035 ± 0.006
V_1 (ml)	1.51 ± 0.07	$1.22 \pm 0.10^{**}$
V_{SS} (ml)	7.13 ± 6.78	4.09 ± 0.55
K_{12} (min^{-1})	0.093 ± 0.046	0.029 ± 0.003
K_{21} (min^{-1})	0.041 ± 0.018	$0.012 \pm 0.001^*$
K_{10} (min^{-1})	0.093 ± 0.010	0.068 ± 0.020
$T_{1/2\alpha}$ (min)	3.4 ± 0.6	$7.1 \pm 1.4^{**}$
$T_{1/2\beta}$ (min)	52.7 ± 46.1	85.0 ± 6.9

* $P < .05$.

** $P < .01$.

CSF disposition of these drugs. The mean total plasma C_{max} of BUP was slightly higher than that of LID ($2.23 \pm 1.25 \mu\text{M}$ versus $1.24 \pm 0.41 \mu\text{M}$, $P = .13$). The T_{max} of BUP was significantly lower than that of LID (1.6 ± 1.5 min versus 3.8 ± 1.1 min, $P < .05$).

The CSF concentrations of BUP and LID were much higher than those in plasma. After epidural administration, the mean ratios between CSF and plasma C_{max} of BUP and LID were about 450 and 880, respectively. The mean CSF bioavailability of epidural BUP and LID was 5.5 and 17.7%, respectively.

Unbound Fraction of BUP and LID in CSF. The unbound fractions at the concentrations of 50, 100, 250, and 500 $\mu\text{g/ml}$ were 39.4, 39.5, 39.7, and 43.7% for BUP and 80.0, 76.3, 79.4, and 73.8% for LID, respectively. The mean unbound fractions of BUP and LID, at a concentration of 100 $\mu\text{g/ml}$, were $39.3 \pm 2.3\%$ and $75.8 \pm 7.7\%$ ($n = 4$), respectively.

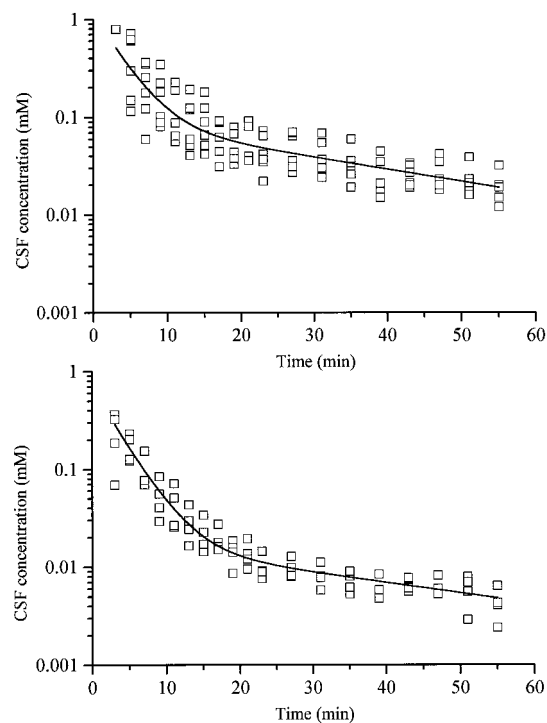


Fig. 2. Measured and population-predicted intrathecal concentrations of bupivacaine (top) and lidocaine (bottom) after separate intrathecal administration of equimolar dose ($0.2 \mu\text{M}$: 0.060 mg of BUP and .049 mg of LID) in rabbits.

Discussion

At present, the evaluation of CSF disposition of drugs has been achieved only by using classical CSF sampling methods, i.e., repeated punctures or sampling through a catheter. The main methodological drawback of these methods is that sam-

TABLE 2

Intrathecal pharmacokinetic parameters (mean \pm S.D.) of BUP and LID after separate intrathecal administration of equimolar dose (0.2 μ M: 0.060 mg of BUP and 0.049 mg of LID) in rabbits

	BUP	LID
$C_{\max\text{-csf}}$ (μ g/ml)	124 \pm 81	65 \pm 19
(mM)	0.43 \pm 0.28	0.28 \pm 0.08
AUC _{csf-it} (mM \cdot min)	7.3 \pm 3.6	3.0 \pm 0.7*
CL _E (ml/min)	0.031 \pm 0.013	0.069 \pm 0.008***
CL _T (ml/min)	0.021 \pm 0.017	0.019 \pm 0.004
V ₁ (ml)	0.18 \pm 0.07	0.30 \pm 0.01*
V _{SS} (ml)	0.62 \pm 0.46	0.91 \pm 0.17
K ₁₂ (min ⁻¹)	0.107 \pm 0.043	0.062 \pm 0.013
K ₂₁ (min ⁻¹)	0.050 \pm 0.005	0.031 \pm 0.003***
K ₁₀ (min ⁻¹)	0.176 \pm 0.027	0.229 \pm 0.033*
T _{1/2α} (min)	2.3 \pm 0.5	2.4 \pm 0.4
T _{1/2β} (min)	24.4 \pm 5.7	28.9 \pm 3.1

* $P < .05$.*** $P < .001$.

TABLE 3

Intrathecal pharmacokinetic parameters (mean \pm S.D.) of BUP and LID after separate epidural administration of equimolar dose (6.9 μ M: 2 mg of BUP and 1.62 mg of LID) in rabbits

	BUP	LID
$C_{\max\text{-csf}}$ (μ g/ml)	325 \pm 248	259 \pm 126
(mM)	1.13 \pm 0.86	1.10 \pm 0.54
T _{max-csf} (min)	5.6 \pm 2.8	7.0 \pm 1.2
AUC _{csf-epi} (mM \cdot min)	13.3 \pm 9.1	19.0 \pm 10.4
CL _E /F _{csf} (ml/min)	0.438 \pm 0.187	0.427 \pm 0.248
V ₁ /F _{csf} (ml)	2.93 \pm 0.27	3.33 \pm 0.46
V _{SS} /F _{csf} (ml)	8.31 \pm 3.65	4.98 \pm 1.20
K ₁₂ (min ⁻¹)	0.097 \pm 0.057	0.031 \pm 0.007*
K ₂₁ (min ⁻¹)	0.055 \pm 0.004	0.067 \pm 0.011*
K ₁₀ (min ⁻¹)	0.148 \pm 0.061	0.122 \pm 0.059
K _a (min ⁻¹)	0.851 \pm 0.256	0.193 \pm 0.062***
T _{1/2α} (min)	3.0 \pm 1.2	4.2 \pm 1.2
T _{1/2β} (min)	23.0 \pm 3.2	16.4 \pm 3.0**

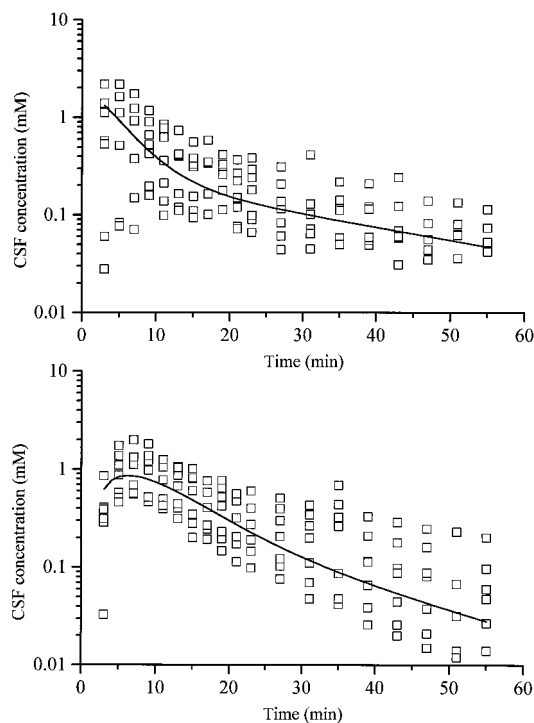
* $P < .05$.** $P < .01$.*** $P < .001$.

Fig. 3. Measured and population-predicted intrathecal concentrations of bupivacaine (top) and lidocaine (bottom) after separate epidural administration of equimolar dose (6.9 μ M: 2 mg of BUP and 1.62 mg of LID) in rabbits.

pling of CSF may interfere with CSF dynamics and disturb the disposition of intrathecally administered drugs. To minimize this problem, sampling frequency has been lowered, precluding suitable pharmacokinetic analysis, especially for drugs displaying rapid disposition steps. This point is of interest because numerous drugs used in anesthetic practice have a high lipophilicity and may have rapid kinetics. Hence, for such studies, microdialysis sampling strategy appears of paramount interest. However, the evaluation of the disposition of drugs in CSF might have some limitations because drug diffusion in CSF is not fast and uniform (Rigler et al., 1991) as it can be seen in blood, even though there is slow fluid dynamics in the intrathecal space.

Microdialysis technique, allowing measurement of unbound drug concentrations, leads to the determination of unbound pharmacokinetic parameters of the pharmacologi-

cally active fraction of drugs. However, we have established that there is a drug binding in CSF that was linear for BUP and LID in the concentration range studied. The mean unbound fraction of BUP was 2 times lower than that of LID. A lowest unbound fraction of BUP in plasma also has been established compared with LID. However, the plasma protein binding of BUP (Denson et al., 1984) and LID (McNamara et al., 1981) is nonlinear in humans. This difference in the binding pattern may be apparent and may result from the high difference (25- to 100-fold) in drug concentration between plasma and CSF studies and/or from species difference in binding.

The microdialysis technique also permitted us to describe the disposition of BUP and LID in the epidural space. After epidural administration, $C_{\max\text{-epi}}$ was lower than C_{inj} for both drugs and doses. There was also a difference in $C_{\max\text{-epi}}$ between BUP and LID (i.e., difference in $C_{\max\text{-epi}}/C_{\text{inj}}$ ratio between BUP and LID), which may be explained by a differential diffusion into epidural fat (Rosenberg et al., 1986). Furthermore, the assumption of a higher distribution of BUP within the epidural space is supported by the 4-fold difference in intercompartmental clearance (CL_T) between BUP and LID, which was close to statistical significance ($P = .06$). The higher epidural elimination clearance (CL_E) of BUP in comparison with LID suggested a more significant uptake of BUP into the systemic circulation and/or into the CSF.

After intrathecal administration of BUP and LID, we showed that the CSF disposition of both drugs displayed a biphasic pattern as described for clonidine in sheep (Castro and Eisenach, 1989) and sufentanil (Hansdottir et al., 1991), morphine, meperidine (Sjöström et al., 1987b), and neostigmine (Shafer et al., 1998) in humans. The biphasic pattern should result from different processes involved in the uptake of intrathecally injected drugs: 1) diffusion along the concentration gradient from CSF through the pia mater into the most superficial portions of the spinal cord (Greene, 1983); 2) access to the deeper areas of the spinal cord through the spaces of Virchow-Robin, which are extensions of the sub-arachnoid space accompanying the blood vessels penetrating the spinal cord from the pia mater (Greene, 1983); 3) drug diffusion through the arachnoid and dura mater and subsequent diffusion in the epidural space (Cohen, 1968); and 4)

uptake into the blood vessels of the pia and dura mater (Vandenabeele et al., 1996).

We found a higher intrathecal CL_E of LID compared with BUP, which is in agreement with the faster systemic uptake of LID after intrathecal administration compared with BUP in humans (Burm et al., 1983). If the CL_E of LID from intrathecal and epidural spaces were close, BUP showed a lower intrathecal CL_E . In contrast to what was observed in the epidural space, CL_I values of BUP and LID in the intrathecal space were very close and lower than the epidural CL_I , highlighting the influence of the epidural fat in epidural drug disposition, especially for BUP. After epidural and intrathecal administrations, the intrathecal apparent elimination half-lives of BUP were close, in contrast to LID, which displayed an unexpected lower intrathecal apparent elimination half-life after epidural administration. It should be noticed that, in contrast to BUP, the CSF concentration profiles of LID were different after intrathecal and epidural administration, the terminal phase occurring later after epidural administration. Moreover, the concentration profiles of LID in CSF after epidural administration and in epidural sites are similar, indicating that the CSF kinetic disposition of LID was influenced by the epidural disposition of this drug, in contrast to BUP.

In the current study, we have estimated the CSF bioavailability of BUP and LID after epidural administration, and our data can be compared to those in the literature. The CSF bioavailability of BUP and LID were higher than those of lipophilic and hydrophilic opioids and close to that reported for clonidine (Table 4). In the current study, the diffusion rate through the meninges (estimated by $T_{max-csf}$) was close to that observed for sufentanil in dogs, but much more rapid than the data usually reported (Table 4). The current investigation showed that the CSF bioavailability of LID, the less lipophilic agent, was around three-times higher than that of BUP.

After epidural administration, drug epidural disposition results from three uptake competing processes: 1) through meninges into CSF, 2) through capillary vessels into systemic circulation, and 3) into epidural fat. Using an ex vivo model, Bernards and Hill (1990) suggested that permeability of BUP and LID through spinal monkey meninges was not different. However, the 4-fold difference in absorption rate constant between BUP and LID was not unexpected, considering the high lipophilicity of BUP. Furthermore, this may suggest that in vitro models should not accurately reflect the in vivo situation because of the relative complexity of the processes involved in the drug disposition in the epidural and intrathecal spaces.

TABLE 4
 F_{csf} and $T_{max-csf}$ of drugs after epidural administration published previously

Drugs	Species	F_{csf}	$T_{max-csf}$	Reference
		%	min	
Morphine	Dog	0.3	5–60	Durant and Yaksh, 1986
	Human	1.9	135	Norberg et al., 1984
	Human	3.6	60–90	Sjostrom et al., 1987a
Meperidine	Human	3.7	15–30	Sjostrom et al., 1987a
Sufentanil	Human	2.7	45	Hansdottir et al., 1995
Clonidine	Sheep	14	11–59	Castro and Eisenach, 1989
BUP	Rabbit	5.5	3–9	This study
LID	Rabbit	17.7	3–9	This study

Although differences in plasma concentrations between two drugs are dependent not only on diffusion but also on distribution and elimination, our data suggested that BUP diffuses more rapidly than LID into systemic circulation (T_{max} 2 times lower for BUP) and suggested that the amount of BUP diffusing into systemic circulation was higher than that of LID (C_{max} 2 times higher for BUP). Indeed, after i.v. administration of LID (4 mg/kg) (Doherty et al., 1995) and BUP (0.6 mg/kg) (Fréville et al., 1996) in rabbits, the mean apparent volume of distribution ($Vd\beta$) of BUP was larger than that of LID (12.4 liters versus 9.4 liters). This would explain, in part, the difference in CSF bioavailability observed after epidural administration and was supported by the fact that epidural clearance of BUP was higher than that of LID. Moreover, this is supported by the fact that an increase in spinal effects of BUP and, to a lesser extent, of LID is observed when a vasoconstrictor such as epinephrine is added after epidural administration (Covino and Wildsmith, 1998).

The second process, which could explain the difference in CSF bioavailability, is the higher partitioning of BUP into epidural fat compared with LID (Rosenberg et al., 1986).

The CSF bioavailability of LID and BUP found in rabbits can be related to the clinical practice of anesthesia in humans. Indeed, the mean doses used epidurally and intrathecally are 440 mg and 90 mg for LID and 140 mg and 15 mg for BUP (Tucker and Mather, 1998), leading to ratios of epidural doses to intrathecal doses of around 5 for LID and 9 for BUP, i.e., to a clinically based bioavailability of approximately 20% for LID and 10% for BUP.

This first in vivo investigation of the spinal disposition of local anesthetics in animals has several pharmacological implications. Although CSF drug concentrations usually are considered as free drug concentrations, because protein CSF concentrations are very low, we have shown that there is a drug binding in CSF fluid. The investigation of the drug binding for local anesthetics in the human CSF should be performed and may explain the large variability of effect after their intrathecal administration, especially for BUP. Even if the diffusion rate through meninges of BUP was higher than that of LID, our investigation showed a higher CSF bioavailability for LID that should result from a difference in the epidural disposition of these drugs. Furthermore, such a model should be of interest to define the basis of the development of drug delivery systems for local anesthetics currently investigated to improve the clinical and toxicological features of epidural local anesthetics.

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