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Ex vivo and *in vivo* diffusion of ropivacaine through spinal meninges: Influence of absorption enhancers

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ABSTRACT

Following epidural administration, cerebrospinal fluid bioavailability of local anesthetics is low, one major limiting factor being diffusion across the arachnoid mater barrier. The aim of this study was to evaluate the influence of absorption enhancers on the meningeal permeability of epidurally administered ropivacaine. Five enhancers known for their ability to increase drug permeability via transcellular and/or paracellular pathways, i.e. palmitoyl carnitine, ethylenediaminetetraacetic acid, sodium caprate, dodecylphosphocholine and pentylglycerol, were tested *ex vivo* on fresh specimen of meninges removed from cervical to lumbar level of rabbit spine following laminectomy and placed in diffusion chambers. Among them, sodium caprate lead to the best permeability improvement for both marker and drug (440% and 112% for mannitol and ropivacaine, respectively) and was therefore selected for *in vivo* study in a sheep model using microdialysis technique to evaluate epidural and intrathecal ropivacaine concentrations following epidural administration. Resulting dialysate and plasma concentrations were used to calculate pharmacokinetic parameters. Following sodium caprate pre-treatment, ropivacaine intrathecal maximal concentration (Cmax) was 1.6 times higher ($78 \pm 16\ \mu g\ ml^{-1}$ vs $129 \pm 26\ \mu g\ ml^{-1}$, $p < 0.05$) but the influence of the absorption enhancer was only effective the first 30 min following ropivacaine injection, as seen with the significantly increase of intrathecal $AUC_{0-30\,min}$ ($1629 \pm 437\ \mu g\ min\ ml^{-1}$ vs $2477 \pm 559\ \mu g\ min\ ml^{-1}$, $p < 0.05$) resulting in a bioavailable fraction 130% higher 30 min after ropivavaine administration. Co-administration of local anesthetics with sodium caprate seems to allow a transient and reversible improvement of transmeningeal passage into intrathecal space.

1. Introduction

Spinally active drugs such as local anesthetics administered epidurally must cross the meninges to reach their sites of action in the spinal cord. The meninges comprise the dura mater and the leptomeninges (arachnoid and pia mater) which have multiple functions and anatomical relationships. Dura mater forms an outer endosteal layer related to the bones of the skull and spine and an inner layer closely applied to the arachnoid mater. The arachnoid mater is known as the principal meningeal barrier (Ummenhofer and Bernards, 1997) with an outer parietal layer impermeable to cerebrospinal fluid (CSF) due to the presence of tight intercellular junctions also limiting drugs diffusion across the meninges (Vandenabeele et al., 1996).

Local anesthetics are known to cross the meninges mainly by diffusion through the arachnoid mater but as these drugs are weak bases, a large fraction of the dose administered may be ionized when local pH is lower than drug pK_a and because this hydrophilic fraction will not cross the meninges via passive diffusion it should be interesting to take advantage of an improved paracellular diffusion in order to improve the low spinal bioavailability of epidurally administered local anesthetics, around 10% (Clement et al., 1999; Rose et al., 2007). Absorption enhancers are compounds which are known to increase the permeability of chemicals transcellularly, or paracellularly via loosening tight junctions. Many compounds such as calcium chelators, surfactants, chitosan or cyclodextrins have been studied for their absorption enhancement potential, *in vitro* on models of intestinal epithelium such as Caco-2 monolayers (Boulenc et al., 1995; Liu et al., 1999; Ward et al., 2000), *ex vivo* on colon samples (Shimazaki et al., 1998) and on meningeal maters (Bernards and Kern, 1996; Ummenhofer and Bernards, 1997) as

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well as *in vivo* on rat nasal respiratory epithelium (Marttin et al., 1999; Illum et al., 1994), on rabbit oral bioavailability (Dos Santos et al., 2003), on rat rectal absorption (Takahashi et al., 1997).

The objectives of this study were to investigate the influence of five absorption enhancers supposed to act on tight junctions, on ropivacaine diffusion through spinal meninges *ex vivo* then *in vivo*. The five enhancers tested were sodium caprate, a constituent of milk fat that was shown to increase tight junction permeability to drugs in rat ileal mucosa (Soderholm et al., 2002); palmitoyl carnitine chloride, a fatty acid derivative of L-carnitine that was found to be effective in increasing transepithelial transport of poorly absorbed drugs both *in vivo* and *in vitro* without altering the integrity of junctional complex (Duizer et al., 1998); dodecylphosphocholine that was found to increase the permeability of paracellular markers such as mannitol with a reversible effect and without disrupting the cell membranes (Liu et al., 1999); ethylenediaminetetraacetic acid (EDTA) known to open the paracellular route via calcium chelation (Tomita et al., 1996) and pentylglycerol, a short-chain alkylglycerol known to affect the physicochemical properties of biological membranes that produced a reversible opening of the blood–brain barrier to hydrophilic drugs following intra-arterial administration in rats thus increasing drug delivery to normal brain and brain tumors by enhanced permeability of tight junctions (Erdlenbruch et al., 2000, 2003). The five previous compounds were screened according to their ability to improve *ex vivo* ropivacaine permeability through rabbit spinal meninges by use of a diffusion chamber system and in a second step the absorption enhancer leading to the best enhancement in drug transport was tested *in vivo* in a sheep model using microdialysis allowing to obtain epidural and intrathecal concentrations.

2. Materials and methods

2.1. Chemicals

Three amide local anesthetics, ropivacaine (Naropeine®), bupivacaine and etidocaine (Astra Zeneca, Rueil Malmaison, France) were used as substance of interest, internal standard of microdialysis and internal standard of HPLC, respectively. The permeation enhancers used, palmitoyl carnitine chloride, sodium salt of EDTA and capric acid (sodium caprate) were obtained from Sigma (St Louis, MO, USA), dodecylphosphocholine was obtained from Avanti Polar Lipids (Alabaster, AL, USA) and pentylglycerol was obtained from Ecole Nationale Supérieure de Chimie de Rennes (Université de Rennes I, UMR 6226). [^{3}H]Mannitol used as hydrophilic radiotracer of paracellular passage (MW 182; specific activity 20 Ci/mmol; radiochemical purity 99%) was obtained from Sigma (St Louis, MO, USA). A mock cerebrospinal fluid (CSF) solution (NaCl 8.2 g/l, $MgCl_2 \cdot 6H_2O$ 0.08 g/l, urea 0.2 g/l, glucose 0.7 g/l, $CaCl_2 \cdot 2H_2O$ 0.3 g/l; pH = 7.38–7.42; 292–298 mOsm/l) was used as medium for the *ex vivo* experiment on isolated meninges and a Ringer's solution (NaCl 8.6 g/l, KCl 0.33 g/l, $CaCl_2 \cdot 2H_2O$ 0.3 g/l, pH 7.0) was used as perfusion fluid during the microdialysis experiments (Clement et al., 2004). All other reagents were of analytical grade.

2.2. In vitro experiment

2.2.1. Tissue preparation

Meningeal maters were obtained from New Zealand albino rabbits weighting 3.0–3.5 kg that were housed individually with free access to food and water in a temperature controlled room ($22 \pm 2\,^\circ$C). The study was achieved according to the guidelines for laboratory animal experiments (French Ministry of Agriculture authorization # B35-238-21). All animals were anesthetized with thiopental then killed with KCl before removal of the meningeal specimens. The entire spinal cords embedded with meninges were revealed by laminectomies from lumbar to cervical level, then removed and both meningeal layers (dura mater and arachnoid) were dissected, kept in their physiological anatomic relationships and placed between two halves of a temperature-controlled plexiglas diffusion chamber with a 0.6 cm^2 connecting port (Costar Corporation, Buckinghamshire, UK).

2.2.2. Flux measurements

Eight milliliters of mock CSF were introduced in fluid reservoirs on both sides of the meningeal tissue. Oxygen (95%) and carbon dioxide (5%) were bubbled in each fluid reservoir to maintain pH and to provide oxygen to the meningeal cells. The temperature was kept at 37 °C during all the experiments. At the beginning of the experiment, 2.6 μmole ropivacaine or 2.6 μmole mannitol hydrogen-labeled radiotracer was added to the donor reservoir on the dura mater side of the diffusion cell, giving an initial donor concentration of 0.32 mM for both ropivacaine and mannitol. The diffusion of the compounds was studied by sampling 50 μl simultaneously in the donor and in the receiver compartments at time 15, 30, 45 and 60 min and replaced with the same volume mock CSF solution in order to maintain volumes. The samples were placed in scintillation vials with scintillation liquid cocktail (Ultima Gold®, Packard Instrument, Downers Grove, IL, USA) to determine mannitol concentration and in HPLC vials for ropivacaine quantification (see Section 2.3.4 below).

At time = 60 min, solutions of absorption enhancers were added to the donor reservoir leading to initial concentrations of 2.5 or 5 mM for palmitoyl carnitine, 10 or 20 mM for sodium caprate, 2.5 or 5 mM for EDTA, 0.75 or 1 mM for dodecylphosphocholine and 10 or 50 mM for pentylglycerol. Then, 50 μl were withdrawn from both reservoirs every 15 min until 195 min. The slope of the line relating concentration in donor reservoir versus time data, i.e. the drug's flux through the meninges, was determined by least-squares linear regression and apparent permeability coefficients (Papp) were calculated using the following equation:

$$\text{Papp} = \frac{(dC/dt)V_r}{C_0 A}$$

where V_r is the volume of the receiver compartment, $(dC/dt)V_r$ is the drug's flux through the meninges (mmol/min), A represents the diffusional area of the meningeal pieces (0.6 cm^2) and C_0 is the initial concentration of the marker compound in the donor compartment (mmol/l). Transport enhancement ratios (R) were calculated from Papp values:

$$R = \frac{\text{Papp}_{(\text{marker compound} + \text{absorption enhancer})}}{\text{Papp}_{(\text{marker compound alone})}}$$

All experiments were carried out in triplicate under sink conditions and measurements were expressed as mean ± SD.

2.3. In vivo study

2.3.1. Animals

The study was performed according to a protocol approved by the Local Committee of Laboratory Investigation and Animal Care of our institution and achieved in accordance with the rules and guidelines concerning the care and the use for laboratory animal experiments (agreement n° B35-238-21). Experiment was performed on 4 non-pregnant Lacaunes ewes (mean age of 2.8 ± 1 years and a mean weight of 55.6 ± 3.4 kg) which were obtained from INRA (Saint Gilles, France, authorization number Fr 35 240 046).

2.3.2. Study design

Throughout the experiment, the animals were anesthetized with 1–2% isoflurane in oxygen/air (50%/50%). The general anaesthesia was induced with an intravenous (i.v.) injection of thiopental (5–8 mg/kg) through a catheter inserted in the right jugular vein. Then, animals were intubated, and ventilation was controlled mechanically (end-tidal CO_2 35 ± 5 mmHg). When the systolic blood pressure (SBP) decreased to less than 80 mmHg, isoflurane administration was reduced, and infusion of 500–1000 ml of hydroxyethyl starch was performed as necessary. A heat lamp was used to maintain sheep body temperature above 37.5 °C which was controlled with an oesophageal thermistor.

After blunt dissection, a small laminectomy with the removal of a piece of ligamentum flavum was performed at L5–L6 level. The insertion of epidural and intrathecal catheters was performed under visual control by a modified Seldinger technique. In the first step, a puncture of the dura mater was performed with a Tuohy needle (1.5 mm external diameter). Then, after control of a free CSF reflux, a guide wire was advanced through the needle over 10 cm into the intrathecal space. By sliding it over the guide wire, the Tuohy needle was removed. The custom made catheter (external diameter of 2.0 mm and length of 120 mm), allowing injections around the tip of microdialysis probe, was advanced along the guide wire. Then, after removing the guide wire, the intrathecal microdialysis probe was inserted through catheter only if a CSF reflux was observed. Epidural catheter was inserted by the same technique in order to put the tip of the catheter vis-à-vis to the one in the intrathecal space. Epidural microdialysis probe was introduced in absence of CSF reflux. At the end of surgery, the laminectomy and punction areas were secured with a drop of cyanoacrylate tissue adhesive (Indermil, Tyco Healthcare, Gosport, UK). Then, a small amount of blood was used to occlude the open surgical site allowing to control the flowing back of liquids (i.e. after local anesthetics injection and during the experiment period).

Each animal received two successive injections of 50 mg ropivacaine hydrochloride in 15 ml of 0.9% sodium chloride (pH = 6.2) through the epidural catheter over a period of 1 min, at $t = 0$ and 150 min. Thirty minutes before the second administration of ropivacaine, 20 mM sodium caprate solution in 10 ml of 0.9% sodium chloride was injected epidurally at 5 ml/min. Epidural and intrathecal microdialysis sampling was achieved according to the following schedule: before administration, every minute during 15 first minutes and then every 5 min till the end of experiment (120 min). A venous catheter was inserted in the left jugular vein for blood sampling, and administration of maintenance fluid. Blood sampling were collected before injection and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 30 min and every 15 min until 120 min.

After the removal of the microdialysis probes the control of CSF reflux in intrathecal space and no in epidural space was performed. The removed catheters confirmed good positioning if they presented no distortions. At the end of the experiment, the animals were euthanized with simultaneous intravenous injection of thiopental, potassium chloride and pancuronium.

2.3.3. Microdialysis

Microdialysis was performed using a CMA 102 microinjection pump coupled to a CMA/20 microdialysis probe (membrane length of 10 mm, shaft length 140 mm, 0.5 mm outer diameter, molecular weight cut-off 20 kDa). Dialysates were collected by dilution using a CMA 142 microfraction collector (CMA Microdialysis, Solna, Sweden). During the experiments, microdialysis probes were perfused at 1 $\mu l\,min^{-1}$ with a solution of internal standard (1 $mg\,ml^{-1}$ bupivacaine in a Ringer solution). After probe insertion in both intrathecal and epidural spaces, an *in vivo* equilibration with determination of relative loss (RL) of internal standard ($n = 10$ for each probe tested) was achieved over a period of 45 min. Due to the high sampling frequency in the *in vivo* experiments, an accurate collection of micro-volume dialysates was achieved by immersion of the prolongator of the outlet tubing of microdialysis probe into 100 μl or 200 μl of a 1 $\mu g\,ml^{-1}$ etidocaine solution for intrathecal or epidural probes, respectively. A collection interval of 1 min during the first 15 min of experiment, and of 5 min during the further experiment allowed sampling of 1 and 5 μl of dialysate, respectively.

Throughout the experiments, the RL of bupivacaine was determined in each sample and used to correct the dialysate concentrations.

Before and after *in vivo* implantation, the probes were tested *in vitro* in order to verify the lack of significant deterioration by comparison with RL of internal standards. The inter-batch variability among microdialysis probes was low. Indeed, the *in vitro* RL of internal standard checked before *in vivo* implantation was 0.49 ± 0.04 ($n = 10$).

2.3.4. Chromatographic analysis and drug assay

Ropivacaine quantification in samples from *in vitro* experiments as well as local anesthetics separation and quantification (in intrathecal or epidural dialysates, and in plasma samples) were carried out using a high-pressure liquid chromatographic method with UV absorbance detection ($\lambda = 205$ nm). Aliquots of 20 or 50 μl (for intrathecal or epidural samples, respectively) of the dialysate dilutions were immediately injected onto the chromatographic system. Ropivacaine and bupivacaine were extracted from plasma according to a previously published method following slight modifications (Reif et al., 1998). Briefly, 0.5 ml plasma sample was alkalinized by 50 μl of 1 M NaOH and 3 ml of n-heptane were added. After horizontal shaking for 3 min and centrifugation (3 min at $3500 \times g$), 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 were injected onto the chromatographic system. The chromatographic system consisted of a Milton Roy model spectromonitor-3 UV detector (LDC Milton Roy, Riviera Beach, FL, USA), a Waters Model 600 pump, a Waters Model 717 automatic injector and a Waters Empower-Pro data acquisition system (Waters Assoc., Milford, MA, USA). 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). In plasma, the lower limit of quantification of ropivacaine (LQ) was set at 3.8 ng/ml. The linearity was checked from 5 to 500 ng/ml ($r^2 > 0.998$). In dialysate the LQ of ropivacaine with a 5 min sampling in a 100 μl dilution was set at 0.8 μg/ml.

2.3.5. Data analysis

A non-compartmental analysis using the software package WinNonlin Pro (Pharsight, USA) was applied to epidural, intrathecal and plasma concentrations after epidural administration. The peak concentration (Cmax), the corresponding time (Tmax) and the last measured concentrations (Clast) of ropivacaine are derived from the raw data. The calculated parameters are the area under concentration–time curves calculated to infinity ($AUC_{0-\infty}$), partial area ($AUC_{0-30\,min}$), volume of distribution at steady state (Vss), clearance (Cl), and elimination half-live ($T_{1/2}\beta$).

Intrathecal, epidural and plasma drug concentrations after the second ropivacaine injection was corrected by subtraction of the residual concentrations resulting from the previous administration. Residual concentrations were extrapolated from the last sample point on the basis of the terminal elimination half-life.

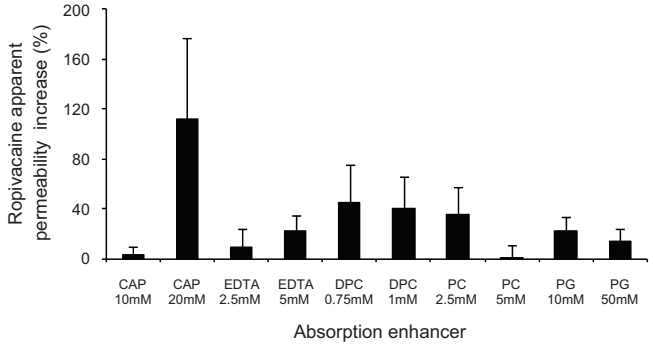

Fig. 1. Ropivacaine apparent permeability increase for each absorption enhancer tested, sodium caprate (CAP), ethylenediaminetetraacetic acid (EDTA), dodecylphosphocholine (DPC), palmitoyl carnitine chloride (PC) and pentylglycerol (PG) (mean ± SD, $n = 6$).

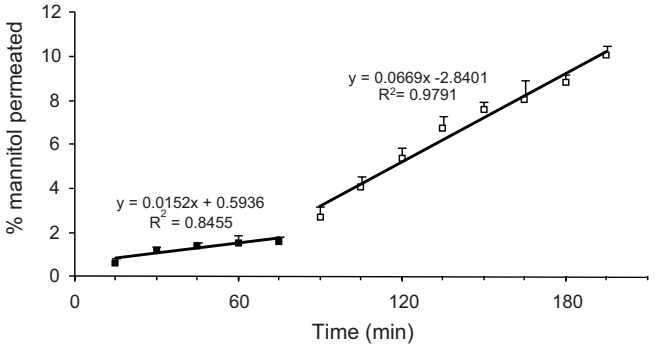

Fig. 2. Percent mannitol permeated through spinal meninges, before (■) and after (□) addition of sodium caprate 20 mM (mean ± SD, $n = 3$).

2.4. Statistical analysis

All data are reported as mean ± SD. Student paired *t* test was performed to assess differences in ropivacaine apparent permeability coefficients before and after absorption enhancer addition and to compare the effect of sodium caprate solution on pharmacokinetics parameters of ropivacaine. A *p* value less than 0.05 was considered as statistically significant.

3. Results

3.1. Influence of absorption enhancers on ex vivo meningeal diffusion

Several conventional absorption enhancers with different mechanisms of action were tested to increase the meningeal diffusion of ropivacaine, and their efficacy was compared (Fig. 1). The rank order of absorption enhancers efficacy was sodium caprate 20 mM ($R = 2.12$) ≫ DCP 0.75 mM ($R = 1.45$) > PC 2.5 mM ($R = 1.35$) > EDTA 5 mM ($R = 1.23$) > AKGP 10 mM ($R = 1.22$). Ropivacaine apparent permeability, 0.111 cm/min reached 0.235 cm/min in the presence of sodium caprate 20 mM (112% increase) and this absorption enhancer was therefore selected to study the meningeal diffusion of the paracellular transport marker mannitol. The mannitol diffusion through rabbit spinal meninges in presence of sodium caprate 20 mM increased by a factor 4.4 compared to mannitol alone (Fig. 2). This result confirmed the potential of sodium caprate to enhance drug transport across meningeal layers, especially for hydrophilic drug as seen with a 4 times higher increase in apparent permeability for mannitol compared to ropivacaine, the latter being more hydrophobic.

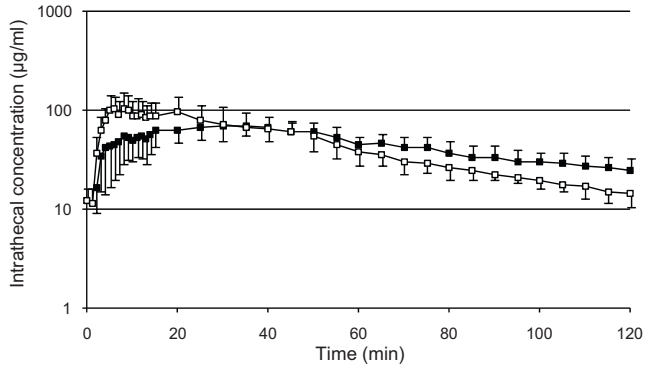

Fig. 3. Mean concentrations of ropivacaine in plasma with (□) and without (■) sodium caprate after epidural administration in sheep (mean ± SD, $n = 4$).

3.2. Influence of sodium caprate on in vivo ropivacaine pharmacokinetics

3.2.1. Intrathecal space pharmacokinetics

Fig. 3 shows mean CSF concentrations of ropivacaine following epidural administration with and without sodium caprate. The CSF non-compartmental pharmacokinetic parameters are listed in Table 1. Sodium caprate increased significantly ropivacaine Cmax in the intrathecal space (78 ± 16 µg/ml vs 129 ± 26 µg/ml, $p < 0.05$).

Table 1
Ropivacaine pharmacokinetic parameters following epidural administration.

	Epidural space		Intrathecal space		Plasma	
	Ropivacaine	Ropivacaine + sodium caprate	Ropivacaine	Ropivacaine + sodium caprate	Ropivacaine	Ropivacaine + sodium caprate
Tmax (min)	2.8 ± 1.3	2.8 ± 1.3	19.3 ± 15.5	9.8 ± 7.0	44 ± 34	6.0 ± 1.4
Cmax (µg/ml)[a]	–	–	78 ± 16	129 ± 26[b]	124 ± 95	171 ± 108
Clast (µg/ml)[a]	101 ± 61	70 ± 45	25 ± 8.2	10 ± 7.1	53 ± 33	69 ± 43
$AUC_{0-\infty}$ (µg min ml^{-1})[a]	58373 ± 29751	40718 ± 19855	7981 ± 2098	5648 ± 2017	18567 ± 11345	38831 ± 35901
$AUC_{0-30\,min}$ (µg min ml^{-1})[a]	26035 ± 7840	15505 ± 3138[b]	1629 ± 437	2477 ± 559[b]	3024 ± 2353	3063 ± 1827
Bioavailable fraction at $t = 30$ min (%)	–	–	22 ± 8.7	51 ± 25[b]	–	–
$T_{1/2}\beta$ (min)	69 ± 35	85 ± 42	68 ± 12	53 ± 47	127 ± 64	351 ± 290
Cl (ml/min)	0.8 ± 0.5	1.2 ± 0.6	–	–	–	–
Vss (ml)	48 ± 24	85 ± 52	–	–	–	–

[a] Plasma concentrations are exprimed in ng/ml and AUC in ng min ml^{-1}.
[b] $p < 0.05$ difference between ropivacaine and ropivacaine with sodium caprate.

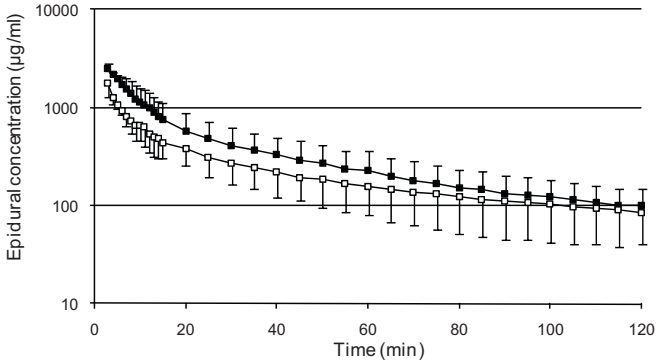

Fig. 4. Mean epidural concentrations of ropivacaine with (□) and without (■) sodium caprate after epidural administration in sheep (mean ± SD, $n = 4$).

$AUC_{0-\infty}$ was not affected by sodium caprate but the $AUC_{0-30\,min}$ increased significantly following sodium caprate pre-treatment ($1629 \pm 437\ \mu g\,min\,ml^{-1}$ vs $2477 \pm 559\ \mu g\,min\,ml^{-1}$, $p < 0.05$) so that ropivacaine bioavailable fraction, increasing from 22 ± 9 to $51 \pm 25\%$, $p < 0.05$, was 130% higher 30 min after ropivavaine administration. All others parameters were not affected.

3.2.2. Epidural space pharmacokinetics

The mean epidural concentration–time profiles of ropivacaine after epidural administration with and without sodium caprate are presented in Fig. 4. The corresponding pharmacokinetic parameters are listed in Table 1. Sodium caprate pre-treatment did not influence the pharmacokinetic parameters of ropivacaine in epidural space. $AUC_{0-\infty}$ was not affected by sodium caprate but the $AUC_{0-30\,min}$ decreased significantly ($26035 \pm 7840\ \mu g\,min\,ml^{-1}$ vs $15505 \pm 3138\ \mu g\,min\,ml^{-1}$, $p < 0.05$).

3.2.3. Plasma pharmacokinetics

Plasma concentrations of ropivacaine after epidural administration with and without sodium caprate are displayed in Fig. 5. The corresponding pharmacokinetics parameters are presented in Table 1. Sodium caprate pre-treatment does not seem to influence ropivacaine plasma pharmacokinetics. Indeed, ropivacaine Cmax in plasma after epidural administration was not affected (124 ± 95 ng/ml vs 171 ± 108 ng/ml for ropivacaine alone and with sodium caprate, respectively). Following sodium caprate pre-treatment, ropivacaine Tmax was shorter (44 ± 34 min vs 6.0 ± 1.4 min) and ropivacaine $T_{1/2}\beta$ was higher (127 ± 64 min vs 351 ± 290 min) although these differences were not significant ($p > 0.05$). Moreover $AUC_{0-30\,min}$ was not significantly modified in presence of sodium caprate ($3024 \pm 2353\ ng\,min\,ml^{-1}$ vs $3063 \pm 1827\ ng\,min\,ml^{-1}$, $p > 0.05$).

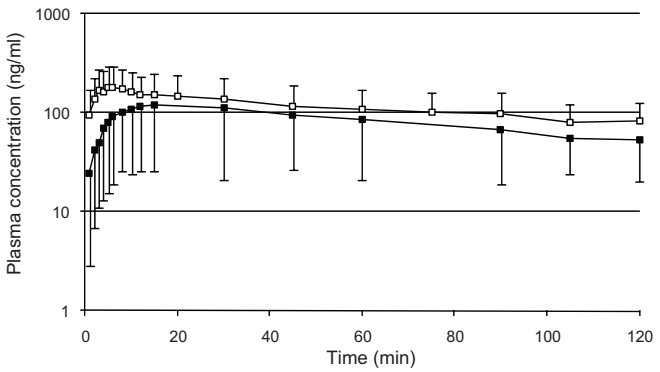

Fig. 5. Mean intrathecal concentrations of ropivacaine with (□) and without (■) sodium caprate after epidural administration in sheep (mean ± SD, $n = 4$).

4. Discussion

The ideal absorption enhancer should be biocompatible, non-toxic with an immediate action and a specific and reversible effect on the junctional complex. The approach for increasing the permeability of drugs via the paracellular pathway may be to co-administer agents that can loosen the tight junctions and hence allow an increase in the transport of poorly bioavailable drugs.

As described previously, the influence of absorption enhancers has been largely documented on substance delivery across the epithelial mucosal lining in lung and gut (Lindmark et al., 1997; Gregory et al., 2003). This has been shown more specifically for sodium caprate used in the current study. Sodium caprate mechanism of action on tight junctions is now well known, increasing the intracellular calcium level to induce contraction of calmodulin-dependent actin filaments (the perijunctional actomyosin ring) which opens the tight junction (Hayashi et al., 1999; Tomita et al., 1995; Shimazaki et al., 1998; Maher et al., 2009). Nevertheless, the resulting increase in epithelial paracellular permeability has been observed with *in vitro* studies with Caco-2 monolayers. For that reason, the possibility that such a mechanism can be exploited to meningeal drug delivery merits investigation. Moreover, only two *in vitro* studies with absorption enhancers were performed through spinal meninges, evaluating the effect of acylcarnitines on monkey transmeningeal flux of hydrophilic drugs classified in hydrophilic (mannitol and morphine) and hydrophobic drugs (sufentanil and bupivacaine) (Bernards and Kern, 1996; Ummenhofer and Bernards, 1997). These studies have shown that acylcarnitines could increase the transmeningeal flux of hydrophilic but not hydrophobic compounds such as bupivacaine *in vitro*. In our study, we selected sodium caprate, an absorption enhancer with a different mechanism of action compared to acylcarnitines that increase calcium intracellular level like sodium caprate but with an action independent of calmodulin (Hayashi and Tomita, 2007). Sodium caprate significantly increased *ex vivo* meningeal permeability for both hydrophilic mannitol and hydrophobic ropivacaine, suggesting that sodium caprate may improve the transmeningeal flux of ropivacaine after epidural administration *in vivo* and showing that hydrophobicity may be a relative notion for ionisable molecules such as local anesthetics when pH is lower than pK_a.

The *in vivo* 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. *In vivo*, several factors may interfere on the intrathecal bioavailability following epidural administration of drugs. Indeed, the epidural space is a fluid-free space containing mainly epidural fat and blood vessels. Three competitive processes are driving the epidural disposition of drugs, i.e. transmeningeal uptake into intrathecal space, uptake into the systemic circulation and distribution into the epidural fat (Clement et al., 2004). Different mechanisms can modulate the balance of these processes. Previous studies have been performed to increase local anesthetics transfer across the spinal meninges, such as a concomitant administration of the vasoconstrictor epinephrine (Ratajczak-Enselme et al., 2007) or the use of a microparticulate local anesthetics-loaded delivery system (Ratajczak-Enselme et al., 2009). In this work, we report the first *in vivo* investigation, with a simultaneous epidural–intrathecal microdialysis, evaluating the influence of the co-administration with an effective adsorption enhancer, sodium caprate on the spinal disposition of ropivacaine after epidural administration in a sheep model. Indeed, sodium caprate has been reported to increase paracellular permeability of drugs widening the intercellular junction by direct action on the membrane (Maher et al., 2009).

Our results indicate that sodium caprate influence the spinal pharmacokinetics and systemic uptake of ropivacaine after epidural administration in sheep. Indeed, intrathecal Cmax and $AUC_{0-30\,min}$ were significantly increased, indicating increased ropivacaine intrathecal absorption during the first 30 min. Moreover, epidural partial $AUC_{0-30\,min}$ was significantly decreased because of the increased ropivacaine intrathecal absorption during the same period of 30 min following local anesthetic administration. Nevertheless, plasma partial $AUC_{0-30\,min}$ was not significantly modified following sodium caprate pre-treatment, showing that sodium caprate effect was not directed to epidural vessels but was only limited to the arachnoid barrier. These results suggest that the effect of sodium caprate on ropivacaine is transient and reversible. This study showed that sodium caprate increased ropivacaine diffusion through spinal meninges *in vivo*. These findings are consistent with the action of caprate on rat blood–brain barrier (Preston et al., 2008), in human keratinocytes and reconstructed epidermis (Kurasawa et al., 2009). In fact, Preston et al. (2008) showed that caprate infusion of 15–25 mM, 2 ml/min for 1 min, conduced to reliable dose-related openings that lasted 1 h, these openings were reversible and produced little or moderate edema, depending on dose.

In future experiments, these results could be improved by modifying the *in vivo* administration scheme of sodium caprate (bolus + perfusion, for example) in order to obtain a sustained opening of tight junctions inducing a prolongation of intrathecal uptake of ropivacaine. In conclusion, the present study shows that the co-administration of ropivacaine with sodium caprate produced a transient and reversible opening of the tight junctions leading to an improvement of the transmeningeal passage into intrathecal space. For further clinical utilisation of such absorption enhancer, spinal toxicity investigation is needed as well as a prolongation of the duration of the pharmacological effect.

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