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Effects of a P-glycoprotein modulator on the pharmacokinetics and distribution of free levobupivacaine and bupivacaine in rats

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ABSTRACT

Pharmacokinetics of free-form levobupivacaine (LB) and free-form racemic bupivacaine (BU) using microdialysis sampling technique were conducted in this study. Three microdialysis probes were implanted in the jugular vein toward the right atrium, brain striatum and bile duct of male Sprague–Dawley rats for concurrently sampling free drug. Effects of P-glycoprotein (P-gp) on the brain distribution and hepatobiliary excretion of LB and BU were examined for the first time after cyclosporine (CsA) administration. LB and BU in samples were determined by HPLC. The blood pharmacokinetics of free LB and free BU were not significantly different. For brain pharmacokinetics, the CsA pretreatment raised significantly the area under curve (AUC) of BU (24.0 ± 5.9 vs. 14.6 ± 4.4 min $\mu\text{g}/\text{mL}$, $p = 0.015$). Brain regions concentrations of LB and BU were significantly higher than plasma concentrations of LB ($p < 0.001$) and BU ($p < 0.001$), respectively. The BU concentration of cerebral cortex was significant higher than that of striatum (6.06 ± 1.03 vs. 4.04 ± 0.90 $\mu\text{g}/\text{mL}$, $p = 0.005$) and hippocampus (6.06 ± 1.03 vs. 4.45 ± 1.07 $\mu\text{g}/\text{mL}$, $p = 0.04$), suggesting that BU might display an uneven brain distribution. For bile pharmacokinetics, LB and BU went through hepatobiliary excretion, and the AUC of BU was significantly higher than that of the LB group (6.6 ± 1.0 vs. 4.6 ± 0.6 min $\mu\text{g}/\text{mL}$, $p = 0.005$). In addition, the pretreatment of CsA significantly reduced the hepatobiliary excretion of BU in terms of AUC (4.4 ± 0.8 vs. 6.6 ± 1.0 min $\mu\text{g}/\text{mL}$, $p = 0.003$). Furthermore, the maximum concentration (C_{max}) of BU diminished significantly as a result of the CsA pretreatment (0.10 ± 0.03 vs. 0.20 ± 0.05 $\mu\text{g}/\text{mL}$, $p = 0.002$). To sum up, enantioselective brain distribution and hepatobiliary excretion of free LB and free BU were observed, and P-gp may relate to the drug transport.

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1. Introduction

Bupivacaine [(±)-1-butyl-N-(2,6-dimethylphenyl)-2-piperidine carboxamide] is the most widely used long-acting local anaesthetic, and is available as a racemic mixture of the R(+)- and S(-)-enantiomers (Casati and Putzu, 2005). Bupivacaine (BU) can be used for epidural anaesthesia in obstetrics (de Barros Duarte et al., 2007) and for the prevention of postoperative pain after major abdominal and orthopedic surgery (Schweitzer and Morgan, 1987). The unbound form in blood R(+)-bupivacaine (dextrobupivacaine) was approximately 6.6%, while S(-)-bupivacaine (levobupivacaine, LB) was around 4.5%, indicating a high plasma protein-binding char-

acteristic of BU (Burm et al., 1994). Recently, it has been found that changed pharmacokinetics of local anaesthetic (e.g. BU and ropivacaine) in pregnant women and postoperative patients due to varied plasma α 1-acid-glycoprotein and drug protein binding (Scott et al., 1997; Tsen et al., 1999; Burm et al., 2000; Veering et al., 2002). In many clinical cases, determination of the total drug concentration does not always give the needed data regarding the free drug, a fraction of drug molecules that are not binding to human serum albumin, lipoprotein, glycoprotein, α , β , and γ globulins, in plasma which is responsible for distribution, elimination, and therapeutic effects (Wright et al., 1996). Therefore, measuring the free drug will provide more straightforward pharmacokinetic information.

Sampling techniques, such as ultrafiltration, equilibrium dialysis and microdialysis, has been utilized to collect the free drug for the pharmacokinetics of BU. The systemic disposition of BU and mepivacaine after epidural administration in patients was determined by ultrafiltration and equilibrium dialysis (Groen et al., 1998). Microdialysis has been used to study the cerebrospinal

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distribution of BU and lidocaine in rabbits (Clement et al., 1998, 1999, 2000). In addition, the release of BU from microspheres and microcapsules has been characterized by microdialysis sampling to assess its local tissue concentrations and pharmacokinetics (McDonald et al., 2002; Kopacz et al., 2003). However, ultrafiltration requires withdrawing plasma, which may cause excessive body fluid loss in small experiment animals. Equilibrium dialysis usually spends relative longer dialysis time, which is prone to drug degradation and bacterial contamination (Tsai, 2003). Microdialysis offers long-term and continuous sampling without net change in fluid volume of small animals and remains good temporal resolution of pharmacokinetic data (Garrison et al., 2002).

To date, there has been no studies compare the free-form pharmacokinetics of LB and BU. Because of its superiority to ultrafiltration and equilibrium dialysis, we applied a microdialysis system for pharmacokinetics of free BU and LB in rat blood, brain and bile in the present study. In addition, the work also revealed the brain regional distribution of total form LB and BU. Finally, the role of P-glycoprotein (P-gp) on the disposition of free LB and BU was examined. P-gp is an ATP-binding cassette (ABC) transporter that has been demonstrated that it plays a significant role in drug disposition (Lin and Yamazaki, 2003; Castagne et al., 2004; Kalvass et al., 2007). ABC transporters like P-gp and breast cancer resistance protein in the blood–brain barrier limit drug distribution to the brain (Kusuhara and Sugiyama, 2001; Begley, 2004; Perriere et al., 2007). P-gp also involved in hepatobiliary excretion of compounds (Garner et al., 2008; Tian et al., 2008; Bansal et al., 2009). Though an *in vitro* study have indicated that BU is a substrate of P-gp (Varma et al., 2003), no *in vivo* result confirms this phenomenon. Therefore, we used cyclosporine (CsA) pretreatment for competitive inhibition of P-gp in rats to examine if there was any significant change on the brain distribution and hepatobiliary excretion of LB and BU.

2. Experimental

2.1. Chemicals and reagents

Bupivacaine, urethane and α -chloralose were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Levobupivacaine hydrochloride (Chirocaine) was obtained from Abbott (Nycomed Pharma AS, Norway). Cyclosporine (Sandimmun) was purchased from Novartis Pharma (Basle, Switzerland). Acetonitrile, triethylamine and other chemicals were of HPLC grade and obtained from E. Merck (Darmstadt, Germany). Water prepared by the Millipore purifying system (Bedford, MA, USA) was used for all preparations.

2.2. Animal preparations

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Yang-Ming University, Taipei, Taiwan. Male specific pathogen-free Sprague–Dawley rats weighing 300–350 g were obtained from the Laboratory Animal Center of the National Yang-Ming University. The animals had free access to food and water. The rat was anaesthetized with urethane 1.0 g/mL and α -chloralose 0.1 g/mL (1 mL/kg, *i.p.*) before surgery. The femoral vein was cannulated for further drug administration, and the rat's body temperature was maintained by a heating pad during the experiment. All rats remained anaesthetized during the experimental period over which the LB and BU concentrations in blood, brain and bile were monitored, and they were euthanized by overdose CO₂ under the anaesthetic after the experimental endpoint.

2.3. Microdialysis probe fabrication and implantation

Microdialysis probes were fabricated in our laboratory and implanted as reported previously (Tsai et al., 2000, 2001; Tsai,

2001). The microdialysis system consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden) and corresponding microdialysis probes located at samplings sites. Briefly, the dialysis membrane (150 μ m outer diameter with a nominal molecular weight cut-off of 13,000; Spectrum Co., Laguna Hills, CA, USA) for blood, brain, and bile are 10 mm, 3 mm and 7 cm in length, respectively. All unions were cemented with epoxy adhesive, and the probes were made at least 24 h prior to use to allow adequate time for the epoxy adhesive to harden. The blood microdialysis probe was located within the jugular vein/right atrium and perfused with an anticoagulant dextrose solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM). Rat bile streamed from the common bile duct cannula into the microdialysis shunt probe, which was perfused with Ringer's solution (147 mM Na⁺, 2.2 mM Ca²⁺, 4 mM K⁺, pH 7.0), and then flowed into the duodenum. The brain microdialysis probe was implanted in the right striatum (coordinates AP –0.2 mm, ML –3.0 mm, DV –7.5 mm) and perfused with Ringer's solution. The perfusion was delivered by a microinjection pump (CMA/100) at a flow rate of 3 μ L/min. The implantation positions of the probes were verified by standard histological procedure at the end of experiments.

2.4. Recovery of microdialysis probe

An *in vivo* relative loss method, which determines the drug dialyzed from the perfusate (the fluid entering the microdialysis probe) into the dialysate (the fluid flowing out of the microdialysis probe) to measure the exchange efficiency of the dialysis membrane in relation to the drug, was utilized to calibrate the recovery as described previously (Elmqvist and Sawchuk, 1997; Wu et al., 2009; Chien et al., 2010). The recovery studies were done on a separate group of rats. The implantation of microdialysis probes was the same as described in the preceding section. Anticoagulant dextrose solution (for blood microdialysis) or Ringer's solution (for brain and bile microdialysis) containing LB or BU was perfused through the probe at the same flow rate (3 μ L/min). Different concentrations (1, 5 and 10 μ g/mL) of LB or BU were used to ensure that the sampling was concentration-independent. We examined the volume of collected samples within the vial to ensure that the probe did not malfunction or leak during the study period. Following a stabilization period of 2 h post-probe implantation, the perfusate (C_{perf}) and dialysate (C_{dial}) concentrations of LB and BU were collected and determined by HPLC. The *in vivo* relative recovery (R_{dial}) of LB and BU across the microdialysis probe was calculated by the following equation:

$$R_{\text{dial}} = \frac{C_{\text{perf}} - C_{\text{dial}}}{C_{\text{perf}}}$$

Microdialysate concentrations (C_m) were converted to free concentrations (C_u) as follows:

$$C_u = \frac{C_m}{R_{\text{dial}}}$$

2.5. Drug administration and sampling

Bupivacaine (3 mg/mL) was prepared in 0.9% (w/v) sodium chloride. Levobupivacaine (Chirocaine) and cyclosporine (Sandimmun) were diluted with 0.9% (w/v) sodium chloride to 3 mg/mL and 20 mg/mL, respectively. Rats were divided into the following groups ($n=5$ for each group) and received drugs intravenously: group 1 treated with LB (3 mg/kg), group 2 pretreated with CsA (20 mg/kg) 10 min before LB (3 mg/kg) administration, group 3 treated with BU (3 mg/kg) and group 4 pretreated with CsA (20 mg/kg) 10 min before BU (3 mg/kg). After a 2 h post-surgical

stabilization period after probe implantation, the drug was administered. The interval for simultaneous sampling at blood, brain and bile was every 10 min. Microdialysis samples were measured by HPLC on the same experimental day.

2.6. Brain regional distribution and protein binding

Two groups of experimental animals ($n = 5$ for each group) under anaesthesia were administered LB (3 mg/kg) or BU (3 mg/kg) separately via the femoral vein. Blood was collected by cardiac puncture at 15 min after administration, and the rat was sacrificed by decapitation. The blood was centrifuged at $6000 \times g$ for 10 min to obtain supernatant plasma, and the brain was removed and split up into cerebral cortex, striatum, hippocampus, cerebellum, brain stem, and the rest of the brain. A 150 μL aliquot of plasma was used to measure the total form concentration of the drug (C_t), and another 150 μL plasma was further centrifuged by an ultrafiltration tube (Centrifree, Millipore, Bedford, MA, USA) at $10,000 \times g$ under 4°C for 10 min to obtain free form drug (C_f) (Han et al., 1999; Shen et al., 2005). The protein-bound ratio (B) of the drug was calculated by following equation:

$$B = \left[\frac{C_t - C_f}{C_t} \right] \times 100\%$$

2.7. Determinations of LB and BU

The chromatographic system consisted of a PM-80 chromatographic pump (BAS, West Lafayette, IN, USA), an off-line injector (CMA/140, Stockholm, Sweden) equipped with a 20 μL sample loop injector (Rheodyne model 7125) and a UV-Vis detector (Soma, Tokyo, Japan) that operated at room temperature ($25 \pm 2^\circ\text{C}$). Separation was achieved on a LiChrosphere 60 RP-select B column (4.6 mm \times 250 mm, 5 μm , Merck). The mobile phase for plasma, brain homogenate, blood dialysate and brain dialysate was comprised of 10 mM potassium dihydrogen phosphate–acetonitrile–triethylamine (67:33:0.01), adjusted to pH 4.3 with H_3PO_4 . The mobile phase for bile dialysate consisted of 10 mM potassium dihydrogen phosphate–acetonitrile–triethylamine (60:40:0.01), adjusted to pH 6.0 with H_3PO_4 . The flow rate was set at 1.0 mL/min for both mobile phases. LB and BU were monitored at the wavelength of 210 nm throughout the experiments. Output data from the detector were integrated using an EZChrom chromatographic data system (Scientific Software, San Roman, CA, USA).

Microdialysis samples were directly injected into the HPLC system. Brain tissue was mixed with a five-fold volume of 50% acetonitrile and homogenated to an even suspension. Plasma and brain homogenate were prepared by protein precipitation using twofold volume of acetonitrile, and 20 μL supernatant obtained by centrifugation at $10,000 \times g$ for 10 min and filtration with 0.22 μm syringe filter was then analyzed by the HPLC method. Calibration curves were prepared by spiking 10 μL standard solution of LB or BU into 50 μL blank plasma to achieve a linear range from 0.15 to 30 $\mu\text{g}/\text{mL}$. The calibration equation was derived by least-square linear regression of the peak area versus the concentrations of LB or BU. The calibration for brain homogenate was generated in the same manner. Extraction recovery was checked by dividing the peak area of the fortified blank matrix with the peak area of the unextracted standard solution $\times 100\%$. Calibration curves were established by using a blank dialysate spiked with a standard solution of LB or BU to generate different drug concentrations ranging from 0.025 to 5 $\mu\text{g}/\text{mL}$ in dialysate. Analytical method validation was performed according to the guidance by US FDA (Guidance for Industry, 2001, Bioanalytical Method Validation).

2.8. Pharmacokinetic and statistical analysis

Pharmacokinetic calculations were performed on each individual animal's data using WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc., Apex, NC, USA). Blood pharmacokinetic parameters were obtained by the two compartment IV bolus modeling, whereas brain and bile pharmacokinetic parameters were obtained by a non-compartmental modeling. The results were presented as the mean \pm standard deviation of each treatment. Statistics were determined using analysis of variance in the SPSS 18.0 program (SPSS Inc., Chicago, USA). Tukey's honestly significant difference test was used for multiple comparisons of variance. A probability value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. HPLC method validation

The peaks of LB and BU were resolved from endogenous interference in the rat blood, brain and bile microdialysis samples, respectively, as shown in Figs. 1 and 2. All calibration curves for quantification of LB and BU possessed coefficient of determination (r^2) greater than 0.995, and inter- and intra-assay accuracy and precision of the analysis were less than $\pm 10\%$. The *in vivo* LB recoveries were $18.7 \pm 0.8\%$ for blood, $11.1 \pm 0.6\%$ for brain, and $72.7 \pm 1.5\%$ for bile microdialysis. The *in vivo* recoveries of BU in various types of microdialysis probes were $18.6 \pm 0.8\%$, $11.7 \pm 0.6\%$, and $72.5 \pm 1.6\%$ for the probes used in blood, brain and bile, respectively. Each value was evaluated by four separate microdialysis probes. Microdialysis samples were calibrated by the probe recovery before pharmacokinetic analysis.

3.2. Pharmacokinetics of free LB and free BU in blood

The aim of our work is to compare the pharmacokinetics of LB and BU, including their brain distribution and bile excretion. Special administration routes (e.g. intrapleural and epidural) usually require skilled experimenters, and incorrect injection sites may cause altered pharmacokinetic situations and complicate the study. Therefore, we chose an intravenous injection via a femoral vein catheter, which is a direct way for comparing the pharmacokinetics of LB and BU. Urethane–chloralose anaesthesia is a common used combination for pharmacokinetic studies and has been considered acceptable for pharmacokinetic–pharmacodynamic studies (Bertera et al., 2009), though we cannot exclude the possibility about the interaction or synergistic effects between urethane and bupivacaine. The dosing of bupivacaine depends on the indications. In general, maximum single doses of bupivacaine in healthy adults should not exceed 175 mg without epinephrine and 225 mg with epinephrine. The dose should not be repeated at intervals of less than 3 h and maximal doses of 400 mg total in 24 h should not be exceeded (MICROMEDEX, 2010). A human equivalent dose (HED) of bupivacaine used in this study is 0.486 mg/kg (approximately 29 mg for a 60-kg adult) according to FDA draft guidelines (Center for Drug Evaluation and Research, 2002). The disappearance of LB and BU in blood visually exhibited a biexponential decline and was described by a two compartmental model following intravenous bolus administration (Figs. 3 and 4). The pharmacokinetic parameters of LB and BU in blood are summarized in Table 1. LB and BU were used clinically, and previous pharmacokinetic reports were conducted mostly by sampling total form drugs. However, plasma protein varied with different physiological states (e.g. parturient or postoperative), influencing the free fraction of highly protein-bound local anaesthetics, such as BU (a binding ratio of 86% found in this work). Free drug monitoring of BU will provide more pre-

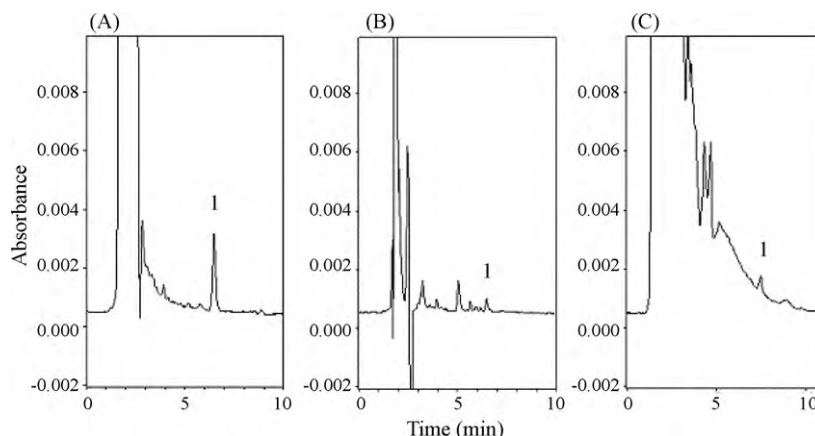


Fig. 1. Representative chromatograms of (A) blood dialysate sample containing levobupivacaine (0.51 µg/mL) collected at 10 min; (B) brain dialysate sample containing levobupivacaine (0.07 µg/mL) collected at 20 min; (C) chromatogram of bile dialysate sample containing levobupivacaine (0.13 µg/mL) collected at 20 min after levobupivacaine administration (3 mg/kg). Peak 1: levobupivacaine.

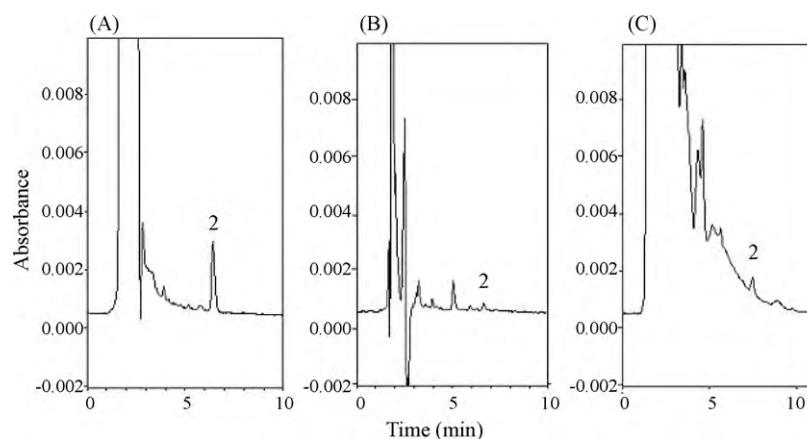


Fig. 2. Representative chromatograms of (A) blood dialysate sample containing bupivacaine (0.46 µg/mL) collected at 10 min; (B) brain dialysate sample containing bupivacaine (0.06 µg/mL) collected at 20 min; (C) bile dialysate sample containing bupivacaine (0.12 µg/mL) collected at 20 min after bupivacaine administration (3 mg/kg). Peak 2: bupivacaine.

cise and useful information. For blood pharmacokinetics, we did not observe significant difference between free LB and free BU. Our results are in accordance with the report that LB has a clinical profile similar to that of BU, and the minimal differences found between the two drugs are chiefly associated with the slightly different anaesthetic potency (Casati and Putzu, 2005). BU contained

equimolar concentrations of the LB and dexbupivacaine, which might reduce the difference between LB and BU. We observed that the AUCs of LB and BU increased after the CsA treatment, though the difference did not reach significant levels (LB vs. CsA + LB, $p = 0.985$; BU vs. CsA + BU, $p = 0.48$). CsA is a cytochrome P450 inhibitor (Lacy et al., 2005), and the CsA treatment might decrease the metabolism

Table 1
Pharmacokinetic parameters of free LB and free BU.

Pharmacokinetic parameters	LB (3 mg/kg)	CsA (20 mg/kg) + LB (3 mg/kg)	BU (3 mg/kg)	CsA (20 mg/kg) + BU (3 mg/kg)
Blood				
$t_{1/2\alpha}$ (min)	6 ± 4	6 ± 1	4 ± 2	6 ± 2
$t_{1/2\beta}$ (min)	47 ± 10	48 ± 17	41 ± 13	39 ± 9
AUC (min µg/mL)	66 ± 9	70 ± 16	82 ± 16	96 ± 17
Cl (mL/min/kg)	46 ± 6	44 ± 11	39 ± 5	32 ± 6
V_{ss} (mL/kg)	2016 ± 655	1875 ± 285	1524 ± 240	1195 ± 124
C_0 (µg/mL)	5.11 ± 3.86	3.59 ± 2.27	6.33 ± 1.93	5.21 ± 0.76
Brain				
C_{max} (µg/mL)	0.56 ± 0.17	0.54 ± 0.10	0.56 ± 0.13	0.70 ± 0.23
AUC (min µg/mL)	12 ± 2	20 ± 4	15 ± 4 ^b	24 ± 6 ^b
Bile				
C_{max} (µg/mL)	0.17 ± 0.02	0.14 ± 0.03	0.20 ± 0.05 ^b	0.10 ± 0.03 ^b
AUC (min µg/mL)	5 ± 1 ^a	4 ± 1	7 ± 1 ^{a,b}	4 ± 1 ^b

Data expressed as mean ± SD. ($n = 5$). C_{max} : maximum concentration; $t_{1/2\alpha}$: distribution half-life; $t_{1/2\beta}$: elimination half-life; AUC: area under the concentration vs. time curve; Cl: clearance; V_{ss} : volume of distribution.

^a Significant difference ($p < 0.05$) between the group of LB (3 mg/kg) and the group of BU (3 mg/kg).

^b Significant difference ($p < 0.05$) between the group of BU (3 mg/kg) and the group of CsA (20 mg/kg) + BU (3 mg/kg).

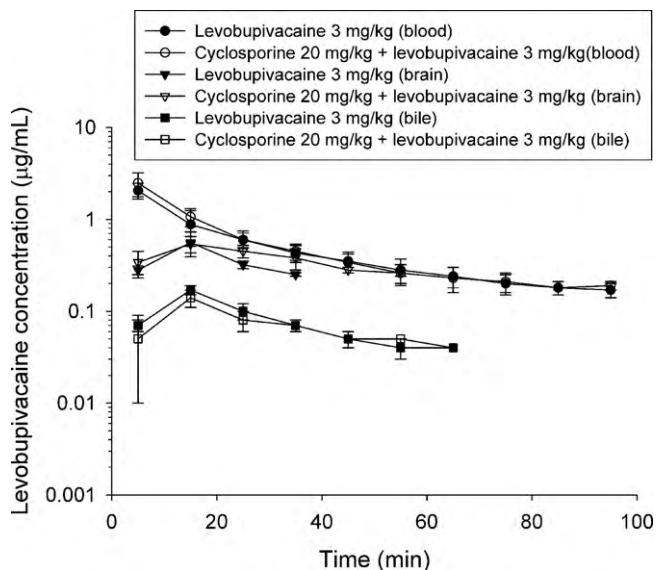


Fig. 3. Concentration–time curves for levobupivacaine in blood, brain and bile after drug administration (3 mg/kg, i.v.) with or without the pretreatment of cyclosporine (20 mg/kg, i.v.). Each group of data is represented as means \pm S.D.

of LB and BU, which accounted for the increased AUCs of LB and BU. In addition, that CsA has a high plasma protein binding property (Yang and Elmquist, 1996; Akhlaghi et al., 1997) might influence the plasma protein binding of BU and increase free-form concentrations and AUCs of LB and BU.

3.3. Pharmacokinetics of free LB and free BU in brain

There was no difference between LB and BU in terms of AUC (12.2 ± 1.9 vs. 14.6 ± 4.4 min $\mu\text{g/mL}$, $p = 0.818$). The CsA pretreatment did not alter the brain distribution of LB (12.2 ± 1.9 vs. 19.8 ± 4.1 min $\mu\text{g/mL}$, $p = 0.057$). However, the CsA pretreatment brought about a significant increase in the AUC of BU (24.0 ± 5.9 vs. 14.6 ± 4.4 min $\mu\text{g/mL}$, $p = 0.015$) as shown in Table 1. The brain distribution of BU has been evaluated in the sheep, but the result was of more relative than absolute value, which was estimated by numer-

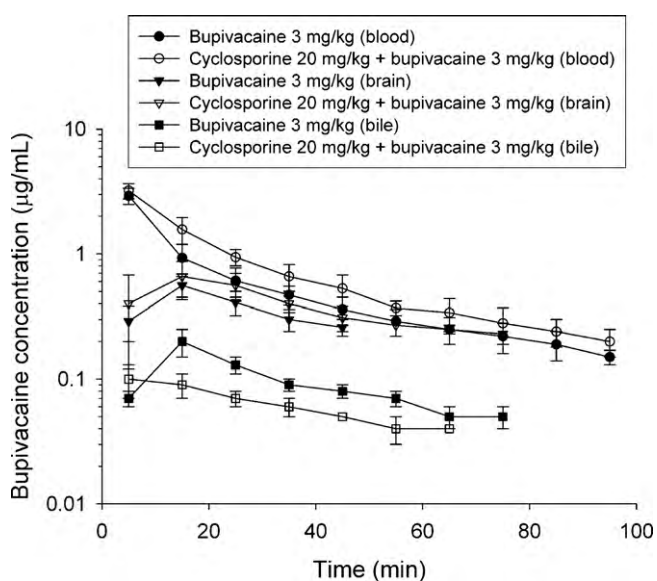


Fig. 4. Concentration–time curves for bupivacaine in blood, brain and bile after drug administration (3 mg/kg, i.v.) with or without the pretreatment of cyclosporine (20 mg/kg, i.v.). Each group of data is represented as means \pm S.D.

Table 2
The brain regional distribution of levobupivacaine and bupivacaine.

	Levobupivacaine	Bupivacaine
Cerebral cortex	5.46 \pm 0.85	6.06 \pm 1.03
Striatum	3.75 \pm 1.73	4.04 \pm 0.90 ^b
Hippocampus	4.60 \pm 0.82	4.45 \pm 1.07 ^b
Cerebellum	4.34 \pm 1.07	4.85 \pm 0.56
Brain stem	4.53 \pm 1.03	4.92 \pm 0.65
The rest of the brain	5.41 \pm 1.10	5.08 \pm 0.66
Plasma	0.96 \pm 0.14 ^a	1.02 \pm 0.20 ^c

Data expressed as mean \pm standard deviation ($n = 5$); the concentration unit for brain tissue: $\mu\text{g/g}$; the concentration unit for plasma: $\mu\text{g/mL}$.

^a Plasma LB concentration was significantly lower ($p < 0.05$) than that of brain regions.

^b Significantly lower BU concentration ($p < 0.05$) than that of cerebral cortex.

^c Plasma BU concentration was significantly lower ($p < 0.05$) than that of brain regions.

ically integrating tissue net fluxes, calculated from the respective products of the measured blood drug concentration and brain blood flows, at each time point sampled over time to obtain calculated net brain content of drug (Mather et al., 1998). It has been demonstrated that quinidine, a P-gp modulator, decreases the threshold of plasma concentration for BU-induced convulsions (Funao et al., 2003). BU interacts with the organic cation/proton antiporter in the brush-border membrane of renal tubular epithelial cells in inhibition of [14C]tetraethylammonium uptake (Gross and Somogyi, 1994). CsA used in this study has been proved to interact with a multitude of active transporters including P-gp (Tamai and Safa, 1990), MRP2 (Kamisako et al., 1999) and OATP (Ho et al., 2006). Thus we cannot rule out the possibility that other transporters might manipulate in the brain distribution of LB and BU.

3.4. Brain regional distribution and protein binding

The drug-plasma protein binding ratios were $86.3 \pm 2.0\%$ and $86.4 \pm 2.1\%$ for LB and BU, respectively. LB and BU concentrations in brain were not different. Brain regions concentrations of LB and BU were significantly higher than plasma concentrations of LB ($p < 0.001$) and BU ($p < 0.001$), respectively, as shown in Table 2. This phenomenon is also found for other local anaesthetics, such as cocaine, exhibiting levels of AUCs at caudate and amygdale twice that of serum (Javaid and Davis, 1993). The BU concentration of cerebral cortex was significant higher than that of striatum (6.06 ± 1.03 vs. 4.04 ± 0.90 $\mu\text{g/mL}$, $p = 0.005$) and hippocampus (6.06 ± 1.03 vs. 4.45 ± 1.07 $\mu\text{g/mL}$, $p = 0.04$), suggesting that BU might display an uneven brain distribution (Table 2).

3.5. Pharmacokinetics of free LB and free BU in bile

Both LB and BU went through hepatobiliary excretion, and the AUC of BU was significantly higher than that of the LB group (6.6 ± 1.0 vs. 4.6 ± 0.6 min $\mu\text{g/mL}$, $p = 0.005$). Hepatobiliary excretion of drugs and endogenous compounds is governed by the cooperation of many transport systems expressed at the sinusoidal and canalicular membrane regions of hepatocytes (Hooiveld et al., 2001). The rat was applied to clarify the role of P-gp on the hepatobiliary excretion of LB and BU, because this animal model possesses similar spectra of substrate specificity and affinity for a number of compounds in rat mdr1a and human MDR1 (Yamazaki et al., 2001). The pretreatment of CsA caused a significant decrease in the AUC of BU (4.4 ± 0.8 vs. 6.6 ± 1.0 min $\mu\text{g/mL}$, $p = 0.003$). Furthermore, the C_{max} of BU was significantly lower after the pretreatment of CsA (0.10 ± 0.03 vs. 0.20 ± 0.05 $\mu\text{g/mL}$, $p = 0.002$) as shown in Table 1. That reduced hepatobiliary excretion by CsA was only observed for BU suggested that P-gp binding affinities of LB and dextrobupiva-

caine might be not the same. Previous studies have discovered that the inhibitory effects of bupivacaine on sarcolemmal adenosine triphosphate-sensitive potassium channels in the cardiovascular system are more potent than that of levobupivacaine (Kawano et al., 2004), and the inhibition of human Kv1.1 potassium channels by BU is more potent than that by LB (Punke and Friederich, 2008). Therefore, it seemed that stereospecific interactions between BU enantiomers and P-gp existed, and resulted in distinct hepatobiliary excretion of LB and BU.

4. Conclusions

In conclusion, no significant difference between free LB and free BU for blood pharmacokinetics was found in our current study. However, enantioselectivity of LB and BU existed, which was evidenced by the fact that the P-gp modulator had greater effects on the brain distribution and hepatobiliary excretion of BU than that of LB.

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References

- Akhlaghi, F., McLachlan, A.J., Keogh, A.M., Brown, K.F., 1997. Effect of simvastatin on cyclosporine unbound fraction and apparent blood clearance in heart transplant recipients. *Br. J. Clin. Pharmacol.* 44, 537–542.
- Bansal, T., Mishra, G., Jaggi, M., Khar, R.K., Talegaonkar, S., 2009. Effect of P-glycoprotein inhibitor, verapamil, on oral bioavailability and pharmacokinetics of irinotecan in rats. *Eur. J. Pharm. Sci.* 36, 580–590.
- Begley, D.J., 2004. ABC transporters and the blood–brain barrier. *Curr. Pharm. Des.* 10, 1295–1312.
- Bertera, F.M., Di Verniero, C.A., Mayer, M.A., Bramuglia, G.F., Taira, C.A., Höcht, C., 2009. Is urethane–chloralose anaesthesia appropriate for pharmacokinetic–pharmacodynamic assessment? Studies with carvedilol. *J. Pharmacol. Toxicol. Methods* 59, 13–20.
- Bupivacaine. MICROMEDEX 1.0 (Healthcare Series) [accessed on June 1, 2010].
- Burm, A.G., Stienstra, R., Brouwer, R.P., Emanuelsson, B.M., van Kleef, J.W., 2000. Epidural infusion of ropivacaine for postoperative analgesia after major orthopedic surgery: pharmacokinetic evaluation. *Anesthesiology* 93, 395–403.
- Burm, A.G., van der Meer, A.D., van Kleef, J.W., Zeijlmans, P.W., Groen, K., 1994. Pharmacokinetics of the enantiomers of bupivacaine following intravenous administration of the racemate. *Br. J. Clin. Pharmacol.* 38, 125–129.
- Casati, A., Putzu, M., 2005. Bupivacaine, levobupivacaine and ropivacaine: are they clinically different? *Best Pract. Res. Clin. Anaesthesiol.* 19, 247–268.
- Castagne, V., Bonhomme-Faivre, L., Urien, S., Ben Reguiga, M., Soursac, M., Gimenez, F., Farinotti, R., 2004. Effect of recombinant interleukin-2 pretreatment on oral and intravenous digoxin pharmacokinetics and P-glycoprotein activity in mice. *Drug Metab. Dispos.* 32, 168–171.
- Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, 2002. Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers. U.S. Food and Drug Administration, Rockville, MD, USA.
- Chien, C.F., Wu, Y.T., Lee, W.C., Lin, L.C., Tsai, T.H., 2010. Herb–drug interaction of *Andrographis paniculata* extract and andrographolide on the pharmacokinetics of theophylline in rats. *Chem. Biol. Interact.* 184, 458–465.
- Clement, R., Malinovsky, J., Le Corre, P., Dollo, G., Chevanne, F., Le Verge, R., 2000. Spinal biopharmaceutics of bupivacaine and lidocaine by microdialysis after their simultaneous administration in rabbits. *Int. J. Pharm.* 203, 227–234.
- Clement, R., Malinovsky, J.M., Dollo, G., Le Corre, P., Chevanne, F., Le Verge, R., 1998. In vitro and in vivo microdialysis calibration using retrodialysis for the study of the cerebrospinal distribution of bupivacaine. *J. Pharm. Biomed. Anal.* 17, 665–670.
- Clement, R., Malinovsky, J.M., Le Corre, P., Dollo, G., Chevanne, F., Le Verge, R., 1999. Cerebrospinal fluid bioavailability and pharmacokinetics of bupivacaine and lidocaine after intrathecal and epidural administrations in rabbits using microdialysis. *J. Pharmacol. Exp. Ther.* 289, 1015–1021.
- de Barros Duarte, L., Moises, E.C., Carvalho Cavalli, R., Lanchote, V.L., Duarte, G., Pereira da Cunha, S., 2007. Placental transfer of bupivacaine enantiomers in normal pregnant women receiving epidural anesthesia for cesarean section. *Eur. J. Clin. Pharmacol.* 63, 523–526.
- Elmqvist, W.F., Sawchuk, R.J., 1997. Application of microdialysis in pharmacokinetic studies. *Pharm. Res.* 14, 267–288.
- Funao, T., Oda, Y., Tanaka, K., Asada, A., 2003. The P-glycoprotein inhibitor quinidine decreases the threshold for bupivacaine-induced, but not lidocaine-induced, convulsions in rats. *Can. J. Anaesth.* 50, 805–811.
- Garner, C.E., Solon, E., Lai, C.M., Lin, J., Luo, G., Jones, K., Duan, J., Decicco, C.P., Maduskuie, T., Mercer, S.E., Gan, L.S., Qian, M., Prakash, S., Shen, H.S., Lee, F.W., 2008. Role of P-glycoprotein and the intestine in the excretion of DPC 333 [(2R)-2-((3R)-3-amino-3-[4-(2-methylquinolin-4-ylmethoxy)phenyl]-2-oxopyrrolidin-1-yl)-N-hydroxy-4-methylpentanamide] in rodents. *Drug Metab. Dispos.* 36, 1102–1110.
- Garrison, K.E., Pasas, S.A., Cooper, J.D., Davies, M.I., 2002. A review of membrane sampling from biological tissues with applications in pharmacokinetics, metabolism and pharmacodynamics. *Eur. J. Pharm. Sci.* 17, 1–12.
- Groen, K., Mantel, M., Zeijlmans, P.W., Zeppenfeldt, B., Olieman, W., Stienstra, R., van Kleef, J.W., Burm, A.G., 1998. Pharmacokinetics of the enantiomers of bupivacaine and mepivacaine after epidural administration of the racemates. *Anesth. Analg.* 86, 361–366.
- Gross, A.S., Somogyi, A.A., 1994. Interaction of the stereoisomers of basic drugs with the uptake of tetraethylammonium by rat renal brush-border membrane vesicles. *J. Pharmacol. Exp. Ther.* 268, 1073–1080.
- Guidance for Industry, 2001. Bioanalytical Method Validation, Center for Drug Evaluation and Research. US Food and Drug Administration, Rockville, MD (May).
- Han, Y.H., Hato, Y., Sugiyama, Y., 1999. Nonlinear disposition kinetics of a novel antifolate, MX-68, in rats. *J. Pharmacol. Exp. Ther.* 291, 204–212.
- Ho, R.H., Tirona, R.G., Leake, B.F., Glaeser, H., Lee, W., Lemke, C.J., Wang, Y., Kim, R.B., 2006. Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* 130, 1793–1806.
- Hooiveld, G.J., van Montfoort, J.E., Meijer, D.K., Muller, M., 2001. Function and regulation of ATP-binding cassette transport proteins involved in hepatobiliary transport. *Eur. J. Pharm. Sci.* 12, 525–543.
- Javaid, J.I., Davis, J.M., 1993. Cocaine disposition in discrete regions of rat brain. *Biopharm. Drug Dispos.* 14, 357–364.
- Kalvass, J.C., Olson, E.R., Pollack, G.M., 2007. Pharmacokinetics and pharmacodynamics of alfentanil in P-glycoprotein-competent and P-glycoprotein-deficient mice: P-glycoprotein efflux alters alfentanil brain disposition and antinociception. *Drug Metab. Dispos.* 35, 455–459.
- Kamisako, T., Leier, I., Cui, Y., Konig, J., Buchholz, U., Hummel-Eisenbeiss, J., Keppler, D., 1999. Transport of monoglucuronosyl and bisglucuronosyl bilirubin by recombinant human and rat multidrug resistance protein 2. *Hepatology* 30, 485–490.
- Kawano, T., Oshita, S., Takahashi, A., Tsutsumi, Y., Tomiyama, Y., Kitahata, H., Kuroda, Y., Nakaya, Y., 2004. Molecular mechanisms of the inhibitory effects of bupivacaine, levobupivacaine, and ropivacaine on sarcolemmal adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Anesthesiology* 101, 390–398.
- Kopacz, D.J., Bernards, C.M., Allen, H.W., Landau, C., Nandy, P., Wu, D., Lacouture, P.G., 2003. A model to evaluate the pharmacokinetic and pharmacodynamic variables of extended-release products using in vivo tissue microdialysis in humans: bupivacaine-loaded microcapsules. *Anesth. Analg.* 97, 124–131 [table of contents].
- Kusuhara, H., Sugiyama, Y., 2001. Efflux transport systems for drugs at the blood–brain barrier and blood–cerebrospinal fluid barrier (Part 1). *Drug Discov. Today* 6, 150–156.
- Lacy, C.F., Armstrong, L.L., Goldman, M.P., Lance, L.L., 2005. Lexi-Comp's Drug Information Handbook International: With Canadian and International Drug Monographs, 13th ed. Lexi-Comp Inc., Ohio.
- Lin, J.H., Yamazaki, M., 2003. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin. Pharmacokinet.* 42, 59–98.
- Mather, L.E., Huang, Y.F., Veering, B., Pryor, M.E., 1998. Systemic and regional pharmacokinetics of levobupivacaine and bupivacaine enantiomers in sheep. *Anesth. Analg.* 86, 805–811.
- McDonald, S., Faibushevich, A.A., Garnick, S., McLaughlin, K., Lunte, C., 2002. Determination of local tissue concentrations of bupivacaine released from biodegradable microspheres and the effect of vasoactive compounds on bupivacaine tissue clearance studied by microdialysis sampling. *Pharm. Res.* 19, 1745–1752.
- Perriere, N., Yousif, S., Cazaubon, S., Chaverot, N., Bourasset, F., Cisternino, S., Declèves, X., Hori, S., Terasaki, T., Deli, M., Scherrmann, J.M., Tamsamani, J., Roux, F., Couraud, P.O., 2007. A functional in vitro model of rat blood–brain barrier for molecular analysis of efflux transporters. *Brain Res.* 1150, 1–13.
- Punke, M.A., Friederich, P., 2008. Lipophilic and stereospecific interactions of amino–amide local anesthetics with human Kv1.1 channels. *Anesthesiology* 109, 895–904.
- Schweitzer, S.A., Morgan, D.J., 1987. Plasma bupivacaine concentrations during postoperative continuous epidural analgesia. *Anaesth. Intensive Care* 15, 425–430.
- Scott, D.A., Emanuelsson, B.M., Mooney, P.H., Cook, R.J., Junstrand, C., 1997. Pharmacokinetics and efficacy of long-term epidural ropivacaine infusion for postoperative analgesia. *Anesth. Analg.* 85, 1322–1330.
- Shen, J., Jiao, Z., Yu, Y.Q., Zhang, M., Zhong, M.K., 2005. Quantification of total and free mycophenolic acid in human plasma by liquid chromatography with fluorescence detection. *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci.* 817, 207–213.
- Tamai, I., Saha, A.R., 1990. Competitive interaction of cyclosporins with the Vinca alkaloid-binding site of P-glycoprotein in multidrug-resistant cells. *J. Biol. Chem.* 265, 16509–16513.

- Tian, X., Swift, B., Zamek-Gliszczynski, M.J., Belinsky, M.G., Kruh, G.D., Brouwer, K.L., 2008. Impact of basolateral multidrug resistance-associated protein (Mrp) 3 and Mrp4 on the hepatobiliary disposition of fexofenadine in perfused mouse livers. *Drug Metab. Dispos.* 36, 911–915.
- Tsai, T.H., 2001. Pharmacokinetics of pefloxacin and its interaction with cyclosporin A, a P-glycoprotein modulator, in rat blood, brain and bile, using simultaneous microdialysis. *Br. J. Pharmacol.* 132, 1310–1316.
- Tsai, T.H., 2003. Assaying protein unbound drugs using microdialysis techniques. *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci.* 797, 161–173.
- Tsai, T.H., Lee, C.H., Yeh, P.H., 2001. Effect of P-glycoprotein modulators on the pharmacokinetics of camptothecin using microdialysis. *Br. J. Pharmacol.* 134, 1245–1252.
- Tsai, T.H., Shum, A.Y., Chen, C.F., 2000. Enterohepatic circulation of chloramphenicol and its glucuronide in the rat by microdialysis using a hepato-duodenal shunt. *Life Sci.* 66, 363–370.
- Tsen, L.C., Tarshis, J., Denson, D.D., Osathanondh, R., Datta, S., Bader, A.M., 1999. Measurements of maternal protein binding of bupivacaine throughout pregnancy. *Anesth. Analg.* 89, 965–968.
- Varma, M.V., Ashokraj, Y., Dey, C.S., Panchagnula, R., 2003. P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. *Pharmacol. Res.* 48, 347–359.
- Veering, B.T., Burm, A.G., Feyen, H.M., Olieman, W.J.H.M.S., Van Kleef, J.W., 2002. Pharmacokinetics of bupivacaine during postoperative epidural infusion: enantioselectivity and role of protein binding. *Anesthesiology* 96, 1062–1069.
- Wright, J.D., Boudinot, F.D., Ujhelyi, M.R., 1996. Measurement and analysis of unbound drug concentrations. *Clin. Pharmacokinet.* 30, 445–462.
- Wu, Y.T., Lin, L.C., Tsai, T.H., 2009. Measurement of free hydroxytyrosol in microdialysates from blood and brain of anesthetized rats by liquid chromatography with fluorescence detection. *J. Chromatogr. A* 1216, 3501–3507.
- Yang, H., Elmquist, W.F., 1996. The binding of cyclosporin A to human plasma: an in vitro microdialysis study. *Pharm. Res.* 13, 622–627.
- Yamazaki, M., Neway, W.E., Ohe, T., Chen, I., Rowe, J.F., Hochman, J.H., Chiba, M., Lin, J.H., 2001. In vitro substrate identification studies for P-glycoprotein-mediated transport: species difference and predictability of in vivo results. *J. Pharmacol. Exp. Ther.* 296, 723–735.