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Pharmacokinetics of Bupivacaine during Postoperative Epidural Infusion

Enantioselectivity and Role of Protein Binding

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Background: **Changing plasma protein concentrations may affect the protein binding and pharmacokinetics of drugs in the postoperative period. This study examined the effect of postoperative increases (in response to surgery) in plasma α_1-acid-glycoprotein (AAG) concentrations on the plasma concentrations of the enantiomers of bupivacaine during continuous epidural infusion of racemic bupivacaine for postoperative pain relief.**

Methods: **Six patients scheduled for total hip surgery with combined epidural and general anesthesia received a bolus dose of bupivacaine (65 mg) followed by constant-rate (8 ml/h) epidural infusion of 2.5 mg/ml bupivacaine for 48 h. Total and unbound plasma concentrations of the enantiomers of bupivacaine and plasma AAG concentrations during the 48-h epidural infusion were determined.**

Results: **Total plasma concentrations of the enantiomers of bupivacaine increased steadily during the infusion ($P < 0.0001$), whereas unbound concentrations did not change after 12 h ($P > 0.1$). Total plasma concentrations of *S*(−)-bupivacaine were higher than those of *R*(+)-bupivacaine ($P < 0.02$), whereas unbound concentrations of *S*(−)-bupivacaine were lower than those of *R*(+)-bupivacaine ($P < 0.002$). AAG concentrations initially decreased, but thereafter increased steadily ($P < 0.0001$). Consequently, free fractions of the enantiomers initially increased and then decreased with time ($P = 0.0002$). Free fractions of *S*(−)-bupivacaine were smaller than those of *R*(+)-bupivacaine ($P = 0.0003$).**

Conclusions: **The study confirmed that the pharmacokinetics of bupivacaine are enantioselective. During postoperative epidural infusion, changing plasma AAG concentrations affect the protein binding of both enantiomers of bupivacaine. Consequently, total plasma concentrations of the enantiomers increase with time, whereas unbound concentrations reach a plateau.**

CONTINUOUS epidural infusion of a local anesthetic, commonly bupivacaine or ropivacaine, either as a single agent or in combination with an opioid, is an established and valuable technique for the prevention of postoperative pain after major abdominal and orthopedic surgery. A concern with this technique is that plasma concentrations of the local anesthetic often accumulate during the postoperative period.[1–4] However, recent studies demonstrated that total ropivacaine concentrations during prolonged (72 h) postoperative epidural infusion increased progressively, whereas unbound ropivacaine concentrations leveled off after approximately 24 h.[5,6] The observations reflected changes in the plasma protein binding of ropivacaine secondary to postoperative increases in the plasma concentrations of α_1-acid-glycoprotein (AAG), which is the major binding protein for local anesthetics, such as ropivacaine and bupivacaine. The findings with ropivacaine presumably also hold for bupivacaine. However, a complicating factor with bupivacaine is that this agent is clinically available as a racemate (50–50% mixture) of two stereoisomers, designated *S*(−)-bupivacaine and *R*(+)-bupivacaine, that are mirror images (called enantiomers). In contrast, ropivacaine, which also exists as two enantiomers [*S*(−)-ropivacaine and *R*(+)-ropivacaine], is clinically available as the pure (100%) *S*(−)-enantiomer. Stereoisomers, including enantiomers, have identical sets of atoms configured in the same groups but in different spatial arrangements. Consequently, they may differ in their interactions with macromolecules that have specific spatial arrangements themselves, such as enzymes, plasma proteins, and receptors. Consequently, stereoisomers can differ in their pharmacokinetics and pharmacodynamics and should actually be considered as different drugs.[7–9] For example, the enantiomers of bupivacaine have been shown to differ in their pharmacokinetics,[10–14] protein binding,[10,11] and toxicity.[15–18] A divergence in their protein binding during postoperative epidural infusion, *e.g.*, as a consequence of changes in plasma AAG concentrations, could complicate the interpretation of both total and unbound plasma concentrations, measured as mixed enantiomers, as has commonly been done in the past.

The primary objective of the current study was to examine the relation between postoperative changes in plasma AAG concentrations, protein binding, and both total and unbound plasma concentrations of the enantiomers of bupivacaine during prolonged constant-rate epidural infusion of racemic bupivacaine for postoperative pain relief. A stereospecific assay was used to quantify the total plasma concentrations and the plasma protein binding of the individual enantiomers. This allowed

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us to study the stereoselectivity in the pharmacokinetics and protein binding as a secondary objective.

Materials and Methods

Patients

The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center (Leiden, The Netherlands), and informed consent was obtained from the patients. Six patients (five women, one man; age, 61–74 yr; weight, 63–74 kg, American Society of Anesthesiologists physical status I–II) scheduled for total hip surgery with combined epidural and general anesthesia were enrolled in the study.

Patients with heart failure or atrioventricular conduction abnormalities and those with severe arteriosclerosis or diabetes were excluded from the study. Also excluded were patients with neurologic or muscle disease, infections in the area of the epidural puncture site, and those with a known history of hypersensitivity or any other reaction to an amide-type local anesthetic.

Anesthetic Procedures

The patients received 10 mg oral temazepam as premedication. In the operating room, monitoring equipment was attached and an intravenous cannula for fluid and drug administration was introduced. A central venous catheter was placed into the superior vena cava *via* the basilic or the cephalic vein of the contralateral arm after local infiltration with 10 mg/ml prilocaine. Subsequently, a blank central venous blood sample (30 ml) was collected. A preload of 500 ml dextrose in saline was administered before the start of the epidural procedure, and the infusion rate was subsequently maintained at 2 ml $\cdot$ kg^{-1} $\cdot$ h^{-1}. The heart rate (from the electrocardiogram) was monitored continuously, and blood pressure was measured at 5–15-min intervals with an automatic device (Accutor 1; Datascope, Helsinki, Finland).

With the patient in the sitting position and after local infiltration of the skin with 10 mg/ml prilocaine, a modified 16-gauge Hustead needle was inserted *via* the L3–L4 interspace using the midline approach. After identification of the epidural space with the loss of resistance to saline technique, an epidural catheter was introduced and advanced 5 cm cephalad. Provided that neither cerebrospinal fluid nor blood was obtained on careful aspiration, a test dose of 3 ml racemic bupivacaine HCl, 5 mg/ml, with epinephrine (5 μg/ml) was injected to exclude an inadvertent subarachnoid or intravascular location of the tip of the epidural catheter. Three minutes later a bolus dose of 10 ml racemic bupivacaine HCl, 5 mg/ml, without epinephrine was injected at a rate of 1 ml/s. After the injection, the patient was placed in the supine horizontal position, and an epidural infusion of 8 ml/h racemic bupivacaine HCl, 2.5 mg/ml, (dose rate, 20 mg/h) without epinephrine was started. This infusion was maintained during 48 h. If the systolic arterial pressure decreased more than 30% below the preanesthetic value or to less than 90 mmHg, 5 mg ephedrine was administered intravenously. Bradycardia was treated with 0.25–0.5 mg intravenous atropine.

Thirty minutes after the epidural injection of bupivacaine, general anesthesia was induced with 2 mg/kg etomidate, 0.01 mg/kg pancuronium, and up to 200 μg fentanyl. After orotracheal intubation, anesthesia was maintained with nitrous oxide–oxygen (66%–33%), isoflurane (0.5–1.5%), and pancuronium as required. If the patient showed signs of inadequate anesthesia during surgery, 50 μg fentanyl was administered. Anesthesia was considered inadequate according to the criteria described by Ausems *et al.*[19] These included an increase in systolic arterial pressure greater than 15 mmHg above the normal blood pressure of the patient, as determined preoperatively; a heart rate greater than 90 beats/min in the absence of hypovolemia; somatic responses, such as bodily movements, swallowing, coughing, *etc.*; and autonomic signs, such as lacrimation, flushing, or sweating. At the end of surgery, general anesthesia was terminated and residual muscle relaxation reversed using atropine and neostigmine, as required.

Postoperative Pain Management

In the recovery room and on the ward, the epidural infusion of bupivacaine was continued. In addition, an intravenous patient-controlled analgesia device (Graseby Model 3300, Watford, United Kingdom) was connected and set to deliver 1-mg morphine bolus doses with a lock-out time of 5 min. No background infusion was given.

Assessments

During the first 30 min after the epidural injection of bupivacaine, bilateral analgesia (defined as absence of a painful sensation to pin prick) levels and motor block scores of the lower limbs were assessed at 5-min intervals. Motor block scores were obtained by asking the patient to increase the extended leg, flex the knee, and flex the ankle and were rated per joint (0 = no, 1 = partial, and 2 = complete blockade). The scores of both extremities were added, giving a maximum score of 12 (complete motor block).

Postoperatively, analgesia levels and motor block scores were examined 4, 8, 12, 24, 36, and 48 h after the start of the epidural infusion. If the upper level of analgesia exceeded T4, the epidural infusion rate was allowed to be decreased with 2 ml/h.

Postoperative visual analog scale (10-cm scale; 0 = no pain, 10 = worst possible pain) scores at rest were obtained at the blood sampling times (see Blood Sampling and Assays). At the same times, the patients were questioned about subjective effects such as itching, ring-

Table 1. Total and Unbound (Free) Plasma Concentrations of the Enantiomers of Bupivacaine and *R*(+)-/*S*(−)-bupivacaine Concentration Ratios

	Total Concentrations (ng/ml)			Unbound Concentrations (ng/ml)		
Time	*S*(−)-bupivacaine	*R*(+)-bupivacaine	*R*(+)/*S*(−) Ratio	*S*(−)-bupivacaine	*R*(+)-bupivacaine	*R*(+)/*S*(−) Ratio
12 h	492 ± 124*	374 ± 72†	0.77 ± 0.06	24 ± 7	26 ± 7	1.10 ± 0.07
24 h	733 ± 224*	582 ± 116†	0.82 ± 0.12	25 ± 11	29 ± 10	1.16 ± 0.11
36 h	926 ± 302*	747 ± 160†	0.83 ± 0.11	27 ± 11	31 ± 11	1.16 ± 0.12
48 h	1,224 ± 359*	956 ± 203†	0.80 ± 0.07	29 ± 10	32 ± 10	1.13 ± 0.07
Effect of time	$P < 0.0001$	$P < 0.0001$	$P > 0.1$	$P > 0.2$	$P > 0.1$	$P > 0.3$
S(−)- *versus* *R*(+)-bupivacaine		$P < 0.02$			$P < 0.002$	

Data are mean ± SD.

* Concentrations at each time differed from the concentrations at all other times ($P < 0.01$). † Concentrations at each time differed from the concentrations at all other times ($P < 0.01$), except 24 *versus* 36 h ($P < 0.05$).

ing in the ears, and tingling under the tongue, and blood pressure and pulse rate were measured. The total morphine consumption during the first 24 h and between 24 and 48 h of epidural bupivacaine infusion were recorded.

Blood Sampling and Assays

Central venous blood samples (5 ml) were obtained for determination of the concentration of the enantiomers of bupivacaine at 10, 20, 30, 40, 50, 60, and 90 min and 2, 4, 6, 12, 18, 24, 30, 36, 42, and 48 h after the start of the epidural infusion. Additional samples (12 ml) were obtained for determination of plasma AAG concentrations and for determination of the degree of protein binding and calculation of the unbound plasma concentrations of the enantiomers of bupivacaine at 12, 24, 36, and 48 h. Blood samples were transferred into heparinized centrifuge tubes. Plasma was separated by centrifugation and stored at −20°C.

Free fractions (f_u) of the enantiomers in the plasma samples were determined in duplicate using equilibrium dialysis at 37°C, as described previously.[20] Free fractions were calculated from the total plasma concentrations (C_p) and concentrations in the dialysate (C_d) by the following equation:

$$f_u = \frac{C_d}{C_p - R.C_d} \times 100\%$$

where R is the ratio of the volumes of dialysate and plasma used in the dialysis (in this study, R = 2). Unbound concentrations were calculated by multiplying free fractions with total plasma concentrations. Free fractions in blank blood samples were determined after spiking the samples with 1,200 ng/ml racemic bupivacaine HCl. Concentrations of *S*(−)-bupivacaine and *R*(+)-bupivacaine in plasma and dialysate were measured with enantioselective high-performance liquid chromatography using modifications of the method described by Vletter *et al.*[21] for analysis of the enantiomers of mepivacaine (see Appendix). Total and unbound plasma concentrations are expressed as free base equivalents.

Data Analysis and Statistical Evaluation

Areas under the total plasma concentration–time curves were determined with the linear trapezoidal rule. Unbound clearances of the individual enantiomers were calculated as $Cl_u = k_0/C_{ss,\,u}$, where k_0 is the epidural infusion rate and $C_{ss,\,u}$ the unbound steady state concentration, *i.e.*, the mean of the concentrations measured after 24-, 36-, and 48-h infusion.

Total (measured after 12-, 24-, 36-, and 48-h infusion) and unbound plasma concentrations of the enantiomers of bupivacaine and their ratios, as well as free fractions of the enantiomers and their ratios, and plasma concentrations of AAG (measured before and after 12-, 24-, 36-, and 48-h infusion) were analyzed using analysis of variance (repeated-measures designs) to examine the effect of time and, when applicable, differences between enantiomers. Where appropriate, multiple comparisons were performed using the Student-Newman-Keuls test. Parameters, derived from the plasma concentration–time curves of *S*(−)- and *R*(+)-bupivacaine, were compared with the paired *t* test. The relation between f_u and AAG concentration was evaluated with simple and multiple (accounting for intersubject variability) linear regression analysis.

Results

Epidural infusion rates were maintained at a constant rate throughout the 48-h study period in all six patients. Total and unbound plasma concentrations of the enantiomers of bupivacaine are presented in table 1 and figure 1. Results of the statistical analyses are included in table 1. Total plasma concentrations of both enantiomers increased steadily with time, but unbound plasma concentrations of the enantiomers did not change significantly with time. Total concentrations of *S*(−)-bupivacaine were higher than those of *R*(+)-bupivacaine,

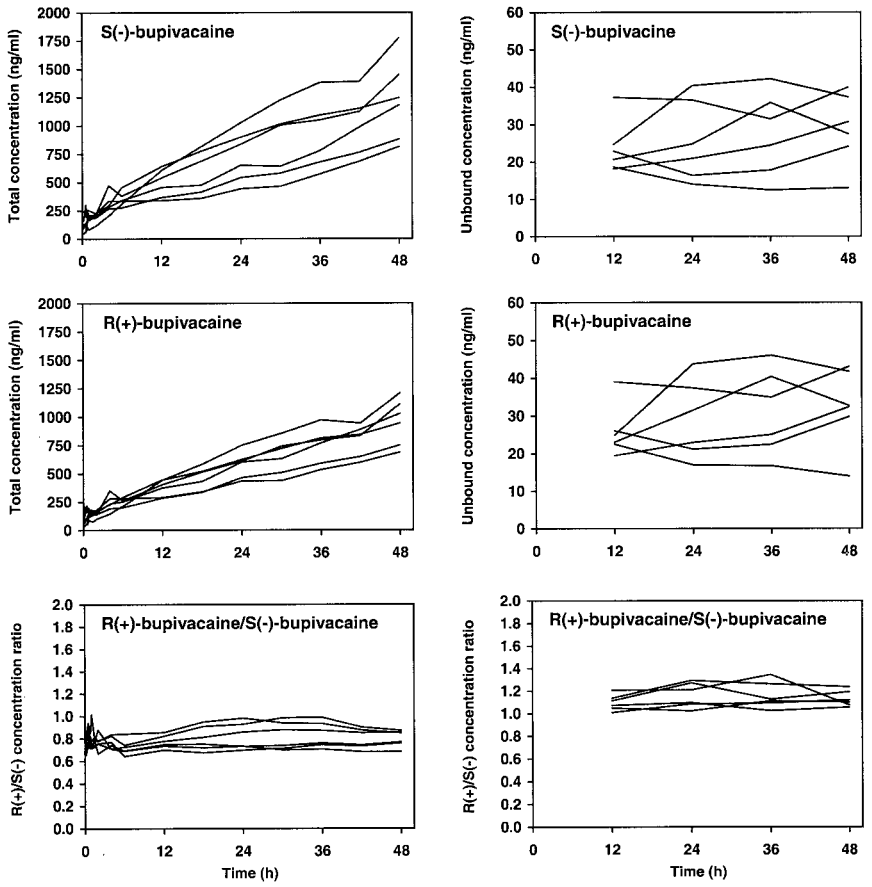

Fig. 1. Individual total (*left*) and unbound (*right*) plasma concentrations of *S*(−)-bupivacaine and *R*(+)-bupivacaine and *R*(+)-bupivacaine/*S*(−)-bupivacaine concentration ratios *versus* time.

whereas unbound concentrations of *S*(−)-bupivacaine were lower than those of *R*(+)-bupivacaine. The ratio of the total as well as the unbound concentrations of the *R*(+) and *S*(−) enantiomers did not change with time. Although the interindividual variations in the total and unbound plasma concentrations of both *S*(−)-bupivacaine (mean coefficients of variation over all time points [CV] = 32 and 37%, respectively) and *R*(+)-bupivacaine (mean CV = 28 and 33%, respectively) were considerable, the interindividual variations in the corresponding *R*(+)-/*S*(−)-bupivacaine concentration ratios were small (mean CV = 10 and 8%, respectively).

Free fractions of both *S*(−)-bupivacaine and *R*(+)-bupivacaine initially increased, but subsequently decreased (table 2). Free fractions of *S*(−)-bupivacaine were smaller than those of *R*(+)-bupivacaine. The ratio of the free fractions of the *R*(+) and *S*(−) enantiomers did not change with time. The interindividual variability in the free fractions of *S*(−)-bupivacaine and *R*(+)-bupivacaine (mean CV = 31 and 32%, respectively) was substantial. However, the interindividual variability in the *R*(+)-/*S*(−)-free fraction ratio was very small (mean CV = 5%).

Plasma concentrations of AAG initially decreased but subsequently increased with time (table 2). Relations

Table 2. Free Fractions of the Enantiomers of Bupivacaine and Plasma Concentrations of AAG

Time	Free Fractions (%)			AAG Concentrations (mg/ml)
	S(−)-bupivacaine	*R*(+)-bupivacaine	*R*(+)/*S*(−) Ratio	
0 h	3.1 ± 0.8	4.6 ± 1.3	1.51 ± 0.03	0.90 ± 0.21†
12 h	5.0 ± 1.4*	7.1 ± 1.9*	1.42 ± 0.03	0.74 ± 0.16†
24 h	3.5 ± 1.1	5.1 ± 1.8	1.43 ± 0.11	0.94 ± 0.16†
36 h	3.1 ± 1.3	4.2 ± 1.7	1.40 ± 0.09	1.18 ± 0.21†
48 h	2.4 ± 0.7	3.4 ± 1.0	1.42 ± 0.09	1.35 ± 0.23†
Effect of time	$P = 0.0002$	$P = 0.0002$	$P > 0.05$	$P < 0.0001$
S(−)- *versus* *R*(+)-bupivacaine		$P = 0.0003$		

Data are mean ± SD.

* Free fractions after 12 h differed from those at all other times ($P < 0.01$). † Concentrations at each time differed from the concentrations at all other times ($P < 0.01$), except 0 *versus* 24 h ($P > 0.05$).

AAG = α_1-acid-glycoprotein.

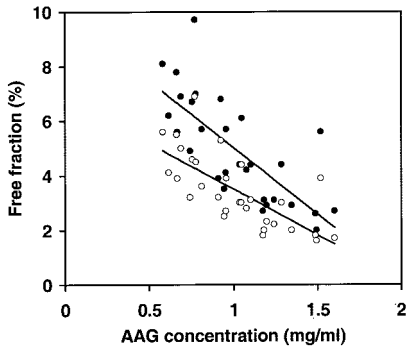

Fig. 2. Measured free fractions of *S*(−)-bupivacaine (open symbols) and *R*(+)-bupivacaine (closed symbols) *versus* plasma α_1-acid-glycoprotein (AAG) concentrations. The corresponding lines were obtained by simple linear regression of the free fractions *versus* the plasma AAG concentrations. Coefficients of determination (R^2) for the free fraction *vs.* plasma AAG concentration relations were 0.52 ($P < 0.0001$) for *S*(−)-bupivacaine and 0.55 ($P < 0.0001$) for *R*(+)-bupivacaine, respectively.

between the free fractions and AAG concentrations are shown in figure 2. Variations in AAG concentrations between subjects and with time explained 72% (R^2 = 0.72) and 75% (R^2 = 0.75) of the variation in the free fractions of *S*(−)-bupivacaine and *R*(+)-bupivacaine, respectively.

Parameters derived from the plasma concentration–time curves of the enantiomers are presented in table 3. Total plasma concentrations of *S*(−)-bupivacaine at the end of the infusion were higher and the areas under the total plasma concentration–time curves of *S*(−)-bupivacaine were larger than those of *R*(+)-bupivacaine, whereas the unbound plasma concentrations at the end of the infusion and unbound steady state concentrations of *S*(−)-bupivacaine were lower than those of *R*(+)-bupivacaine. Unbound plasma clearances of *S*(−)-bupivacaine were higher than those of *R*(+)-bupivacaine.

Clinical Effectiveness

Upper levels of analgesia after 30 min ranged from T9 to T3. Motor block scores after 30 min ranged from 4 to 9. Postoperatively, median upper analgesia levels gradually decreased from T4 after 4 h to L1 after 24 h and L3–L4 after 48 h. Median motor block scores at these times were 12, 5.5, and 3.5, respectively. With few exceptions, visual analog scale scores were less than 3. Only one patient required postoperative morphine (13 mg during the first 24 h, 12 mg during the second 24 h). No signs of central nervous system toxicity were observed at any time.

Discussion

Several studies have shown that plasma concentrations of bupivacaine during long-term postoperative administration increase steadily and may reach concentrations that are in the putative toxic range.[1–4] However, in the same studies, overt toxicity did not occur, although one study showed transient excitation during the initial recovery period.[2] The observations indicated that, during the postoperative period, the relation between the plasma concentration and the risk of systemic toxicity is obscured. Factors that could play a role in this respect are the enantioselectivity in the pharmacokinetics of bupivacaine and postoperative changes in the plasma protein binding of (the enantiomers of) bupivacaine.

So far, all reports of the plasma concentrations of bupivacaine during postoperative epidural administration were based on measurements of mixed enantiomers. However, there is now ample evidence that the pharmacokinetics of bupivacaine in humans are enantioselective.[10–14] In addition, the enantiomers of bupivacaine have been shown to differ in their systemic toxicity.[15–18] If the relative contribution of each enantiomer to the total or the unbound plasma concentration (*i.e.*, the concentration ratio) would vary strongly between subjects or over time, this would complicate the interpretation of plasma concentrations, measured as mixed enantiomers, in relation to the risk of systemic toxicity. In this respect it is reassuring that the current study, although confirming the enantioselectivity of the pharmacokinetics of bupivacaine, also showed that both the interindividual and intraindividual (*i.e.*, the time-depen-

Table 3. Parameters Derived from the Plasma Concentration–Time Curves

	S(−)-bupivacaine	*R*(+)-bupivacaine	*R*(+)/*S*(−) Ratio	*P*
Total plasma concentrations				
$C_{end\ infusion}$ (ng/ml)	1,224 ± 359	956 ± 203	0.80 ± 0.07	< 0.02
AUC_{0-48} ($\mu g \cdot ml^{-1} \cdot h$)	34 ± 9	26 ± 5	0.80 ± 0.09	< 0.02
Unbound plasma concentrations				
$C_{u,\ end\ infusion}$ (ng/ml)	31 ± 15	35 ± 16	1.13 ± 0.07	< 0.01
$C_{ss,\ u}$ (ng/ml)*	28 ± 11	32 ± 11	1.15 ± 0.08	< 0.002
Cl_u (l/min)	6.2 ± 2.9	5.3 ± 2.3	0.87 ± 0.06	< 0.05

Data are mean ± SD.

* Mean of the concentrations measured after 24, 36, and 48 h infusion.

$C_{end\ infusion}$ = total plasma concentration after 48 h infusion; AUC_{0-48} = area under the plasma concentration–time curve between 0 and 48 h; $C_{u,\ end\ infusion}$ = unbound plasma concentration after 48 h infusion; $C_{ss,\ u}$ = unbound steady-state concentration; Cl_u = unbound plasma clearance.

dent) variabilities in the total and unbound concentration ratios are small compared with the corresponding variabilities in the absolute concentrations (fig. 1). Similar small variabilities in the concentration ratios have also been observed in previous studies from our institution.[10,11] In fact, the overall mean (over all subjects and times) total *R*(+)-/*S*(−)-bupivacaine concentration ratios, determined over an 8-h period after intravenous infusion in healthy male volunteers (mean = 0.78, CV = 15%),[7] a 24-h period after a single epidural injection in surgical patients (mean = 0.79, CV = 10%)[8] and during a 48-h epidural infusion (this study: mean = 0.79, CV = 10%) are virtually identical. The corresponding overall mean unbound *R*(+)-/*S*(−)-bupivacaine concentration ratio in the current study was 1.14 (CV = 8%). Comparable data were not obtained in the previous studies, because protein binding was only determined in samples collected shortly before the administration of bupivacaine. However, the *R*(+)-/*S*(−)-bupivacaine free fraction ratios measured in the current study showed minimal interindividual and intraindividual variability (overall mean = 1.44, CV = 6%) and were comparable with the mean values measured shortly before the intravenous administration (mean = 1.50, CV = 6%) and epidural injection (mean = 1.57, CV = 15%) in the previous studies. Therefore, although the numbers of volunteers or patients included in the three studies (n = 6–10) were rather small, the findings appear to be very consistent and strongly suggest that the relative contribution of each enantiomer to both the total and unbound plasma concentrations is little or not dependent on the subject, gender, age, route of administration, and time after the (start of the) administration. Therefore, plasma concentrations, measured previously as mixed enantiomers, are still evaluable, and enantioselectivity in the pharmacokinetics of bupivacaine in itself cannot explain the lack of toxic reactions with the high plasma concentrations observed during long-term epidural administration in some studies.

A more plausible explanation for the latter discrepancy is that unbound plasma concentrations (that are generally believed to be more closely related to the toxic effects) are not increased in proportion to total plasma concentrations during the postoperative period.[22] This, in turn, can be explained by the changes in plasma protein binding, secondary to postoperative changes in plasma AAG concentrations. AAG, also known as orosomucoid, is a so-called acute-phase protein that is sensitive to stressful situations, including surgical trauma.[23] Measurements of AAG concentrations after major surgical procedures usually showed an initial decrease (presumably reflecting postoperative blood loss and fluid replacement) during the first hours, followed by a progressive increase during the next 2–12 days.[5–6,24–26] In addition to AAG concentrations, Wulf *et al.*[25] examined the plasma protein binding of bupivacaine during long-term epidural administration (intermittent injections). In their study, increased AAG concentrations on the fourth compared with the first postoperative day were accompanied by decreased free fractions of bupivacaine (measured as mixed enantiomers), as would be expected. However, plasma concentrations after a 25-mg epidural bolus injection on the fourth postoperative day were lower than those on the first postoperative day. This is in contrast with theoretical expectations. Considering that bupivacaine is predominantly removed from the blood by biotransformation in the liver and that the associated hepatic extraction ratio is small, pharmacokinetic theory predicts that a decrease in free fraction will result in a decrease in the total plasma clearance and, consequently, elevated total plasma concentrations on the fourth postoperative day.[22,27] Wulf *et al.*[25] also reported that unbound bupivacaine concentrations were always less than 100 ng/ml, but no information was given with respect to the exact values or the effect of time.

The results of the current study are in keeping with pharmacokinetic theory and are also in close agreement with observations from a study in cholecystectomy patients that were given 3-h intravenous bupivacaine infusions before and 72 h after surgery.[22] In that study, total bupivacaine (mixed enantiomers) concentrations were almost doubled after surgery, whereas unbound concentrations were similar before and after surgery. Furthermore, the results of the current study are in agreement with those from two recent studies that showed steadily increasing total and relatively constant unbound ropivacaine concentrations during continuous postoperative epidural infusions of that agent.[5,6]

Estimates of the threshold plasma concentrations of bupivacaine (total concentrations, measured as mixed enantiomers) that are associated with the onset of central nervous system toxicity in humans range from 2 to 4 μg/ml.[22] Unbound threshold concentrations and threshold concentrations of the individual enantiomers have been poorly investigated. In one study that compared the effects of levobupivacaine [which contains 100% *S*(−)-bupivacaine] and racemic bupivacaine during a constant-rate infusion in healthy volunteers, the concentration of levobupivacaine at the termination of infusion, when most subjects experienced mild to moderate central nervous system symptoms, was 2.62 μg/ml.[18] The corresponding concentration of bupivacaine (measured as mixed enantiomers) was 2.25 μg/ml. Protein binding was not examined in that study. However, in another study in volunteers, the free fractions of *S*(−)-bupivacaine and *R*(+)-bupivacaine averaged 6.6 and 4.5%, respectively. Based on these numbers, rough estimates of the unbound toxic threshold concentrations of levobupivacaine and bupivacaine (mixed enantiomers) are 175 ng/ml and 125 ng/ml, respectively. In the current study, the sum of the unbound plasma concentrations of *S*(−)-bupivacaine and *R*(+)-bupivacaine ranged

from 32 to 75 ng/ml (mean, 56 ng/ml) between volunteers, which suggests that with the current infusion scheme there is still a twofold to threefold safety margin.

In conclusion, the current study demonstrated that total plasma concentrations of the enantiomers of bupivacaine during constant-rate postoperative epidural infusion of racemic bupivacaine increase progressively, whereas the corresponding unbound concentrations do not increase proportionally but reach a plateau after approximately 12–24 h. The progressive increase in the total plasma concentrations reflects postoperative changes in the protein binding of the enantiomers, secondary to changes in the plasma concentrations of AAG. The study, while confirming the enantioselectivity of the protein binding and pharmacokinetics of bupivacaine, also demonstrated that the interindividual and intraindividual variabilities in the protein binding, as well as in the total and unbound plasma concentrations ratios of *R*(+)-bupivacaine and *S*(−)-bupivacaine, are very small. These observations allow retrospective evaluation of both total and unbound plasma concentrations, previously measured as mixed enantiomers.

Appendix

Determination of the Concentrations of Bupivacaine Enantiomers in Plasma and Dialysate

Concentrations of the enantiomers of bupivacaine in plasma and dialysate were determined with liquid–liquid extraction and enantioselective high-performance liquid chromatography using modifications of the method described by Vletter *et al.*[21] for analysis of the enantiomers of mepivacaine. After addition of the internal standard (ropivacaine, 437 ng/ml plasma or 20 ng/ml dialysate), 0.5-ml plasma aliquots or 1-ml dialysate aliquots were extracted with 5 ml n-pentane. After evaporation of the organic phase on a water bath at 40°C in a gentle stream of nitrogen, the residue was dissolved in 150 μl of the mobile phase.

The high-performance liquid chromatography system consisted of a Gynkotec LPG pump, a Midas auto-injector, including a column thermostat, an SF 757 Absorbance detector equipped with a 12-μl flow cell (all Separations Analytical Instruments, Hendrik Ido Ambacht, The Netherlands), a stainless steel guard column (10 × 4.0 mm) prepacked with 40 μm AAG and a stainless steel analytical column (100 × 4.0 mm) prepacked with 5 μm AAG (both J. T. Baker, Deventer, The Netherlands), a Chromeleon chromatography data system (Gynkotec, Germering, Germany), and a Compaq Pentium 2 computer (Compaq, Houston, TX).

Chromatography was performed in the reversed-phase mode. The column temperature was 30°C. The absorption wavelength of the detector was set at 210 nm with a detector time-constant of 0.5 s. For analysis of plasma samples, the mobile phase consisted of 12% acetonitril (vol/vol) and 88% buffer solution (vol/vol), containing 14 g $Na_2HPO_4.10H_2O$ per liter, and was adjusted to pH 6.8 with phosphoric acid. For analysis of dialysate samples, the mobile phase consisted of 16% acetonitril (vol/vol) and 84% buffer solution. The flow rate of the mobile phase was 1.1 ml/min.

With plasma samples, the retention times were 7.3, 9.2, and 10.7 min for ropivacaine, *R*(−)-bupivacaine, and *S*(+)-bupivacaine, respectively. With dialysate samples, the corresponding retention times were 4.5, 5.2, and 5.9 min. No endogenous compounds were found to interfere with the assays. The detection limits, defined as a signal-to-noise ratio of 3, were 4 ng/ml plasma and 1 ng/ml dialysate for each enantiomer. Interday coefficients of variation for determinations in plasma (n = 6) were 8% and 12% for *R*(+)-bupivacaine and *S*(−)-bupivacaine at concentrations less than 100 ng/ml and 4–8% for both enantiomers at concentrations ranging from 143 to 2,280 ng/ml. Interday coefficients of variation for determinations in dialysate (n = 3) were 2–6% for *R*(+)-bupivacaine and 1–6% for *S*(−)-bupivacaine over the concentration range 11.4–71.2 ng/ml. The recoveries of *R*(+)-bupivacaine and *S*(−)-bupivacaine from plasma were 78 ± 4 (mean ± SD) and 77 ± 3%, respectively, over the concentration range of 11.4–2280 ng/ml. The recovery of ropivacaine was 81 ± 5%.

Calibration curves were obtained by weighted least-squares linear regression analysis (weight factor $1/y^2$) of the enantiomer/ropivacaine peak area ratio *versus* the concentration of that enantiomer. Calibration lines were linear in the investigated range (11.4–2280 ng/ml plasma, 8 calibration points, and 11.4–114 ng/ml dialysate, 5 calibration points) with correlation coefficients (r) varying from 0.995 to 0.999 for determinations in plasma and from 0.997 to 0.999 for determinations in dialysate.

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