Br J clin Pharmac 1994; **38**: 125–129

Pharmacokinetics of the enantiomers of bupivacaine following intravenous administration of the racemate

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1 The pharmacokinetics of R(+)-bupivacaine and S(−)-bupivacaine were investigated following a 10 min intravenous infusion of the racemate (dose 30 mg) in 10 healthy males.

2 The fractions unbound of R(+)- and S(−)-bupivacaine in pre-dose plasma were determined for each subject after *in vitro* addition of rac-bupivacaine (concentration of each enantiomer: approximately 300 ng ml^{-1}).

3 The total plasma clearance of R(+)-bupivacaine (mean ± s.d.: 0.395 ± 0.076 l min^{-1}) was greater ($P < 0.0001$) than that of S(−)-bupivacaine (0.317 ± 0.067 l min^{-1}). The volumes of distribution of R(+)-bupivacaine at steady state (84 ± 29 l) and during the terminal log-linear phase (117 ± 47 l) were larger ($P < 0.0002$) than those of S(−)-bupivacaine (54 ± 20 l and 71 ± 34 l, respectively). The terminal half-life (210 ± 95 min) and mean residence time (215 ± 74 min) of R(+)-bupivacaine were longer than those of S(−)-bupivacaine (157 ± 77 min, $P < 0.01$, and 172 ± 55 min, $P < 0.02$, respectively).

4 The free percentage of R(+)-bupivacaine (6.6 ± 3.0 %) was greater ($P < 0.0002$) than that of S(−)-bupivacaine (4.5 ± 2.1 %).

5 The plasma clearance of unbound R(+)-bupivacaine (7.26 ± 3.60 l min^{-1}) was smaller ($P < 0.01$) than that of S(−)-bupivacaine (8.71 ± 4.27 l min^{-1}). Volumes of distribution based on unbound R(+)-bupivacaine concentrations (Vu_{ss}: 1576 ± 934 l; Vu: 2233 ± 1442 l) did not differ from those of S(−)-bupivacaine (Vu_{ss}: 1498 ± 892 l; Vu: 1978 ± 1302 l).

6 The enantioselective systemic disposition of bupivacaine can to a large extent be attributed to differences in the degree of plasma binding of the enantiomers.

Keywords bupivacaine enantiomers pharmacokinetics protein binding

Introduction

Bupivacaine is an aminoacylaniline type local anaesthetic that is widely used for various local and regional anaesthetic procedures. Since it contains a chiral carbon centre it exists as two enantiomers, R(+)- and S(−)-bupivacaine. Whereas the drug is used clinically as the racemate, there is increasing evidence of enantioselectivity with respect to nerve block characteristics, systemic toxicity and pharmacokinetics [1-7]. Recent studies have shown that the plasma concentrations of S(−)-bupivacaine following interpleural and extrapleural administration of rac-bupivacaine in man are generally higher than those of R(+)-bupivacaine [8–9; Groen *et al.*, unpublished observations]. However, it is not clear whether these differences are due to enantioselective systemic absorption or systemic disposition, or both. This study compared the pharmacokinetics of R(+)- and S(−)-bupivacaine following intravenous administration of rac-bupivacaine in healthy subjects. In addition, it examined the role of plasma binding.

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Methods

Subjects

Ten non-smoking males, aged 19–26 years, body weight 73–92 kg, participated in the study after giving oral informed consent. The study protocol and volunteer information leaflet were approved by the Committee on Medical Ethics of the University Hospital Leiden. The volunteers were judged healthy on the basis of their medical history, physical examination and laboratory tests, including ECG, blood pressure, pulse frequency, urine analysis, haematology and serum biochemistry. None was taking medical or non-medical drugs. They refrained from food and drinks (except water) from midnight prior to the experiment until 3 h after drug administration, when they received a light lunch.

Study design

After arrival of the volunteers and measurement of the blood pressure, a cannula for blood sampling was introduced into an antecubital vein and 30 ml blood were collected. A second cannula for drug infusion was introduced into an antecubital vein in the contralateral arm. Rac-bupivacaine (30 mg rac-bupivacaine.HCl) was then infused at a constant rate in 10 min. The infusion always took place between 09.00 and 12.00 h. Blood samples for determination of the plasma concentrations of the enantiomers were taken at 2, 5, 10, 15, 20 and 30 min after the start of the infusion, subsequently at 15 min intervals until 120 min, at 30 min intervals until 180 min and then hourly until 8 h after the start of the infusion. Blood pressures and pulse rate were measured before, and 5, 10, 15, 30, 45, 60 and 480 min after starting the infusion.

Laboratory methods

Blood samples were centrifuged to obtain plasma, which was stored at −20°C until assayed. The degree of plasma binding of the enantiomers of bupivacaine was determined by adding rac-bupivacaine to blank blood samples. A volume of approximately 5.5 ml of blank sample was taken and and the pH of the sample was adjusted to 7.4 by adding 8 % (w/v) phosphoric acid. Subsequently, 30 µl of a stock solution of bupivacaine hydrochloride in water were added, resulting in a concentration of approximately 600 ng ml^{-1} (300 ng ml^{-1} of each enantiomer). The sample was then warmed to 37° C for 3 h in a water bath. Two 0.5 ml samples were then taken for determination of the total plasma concentrations of R(+)-bupivacaine and S(−)-bupivacaine and four 1 ml samples were subject to ultrafiltration (2000 *g* for 35 min) at 37° C in a thermostated centrifuge (Hettich Universal 2S, Hettich, Tuttlingen, Germany) equipped with a fixed angle (35°) rotor. The Amicon® micropartition system, used for ultrafiltration, was equipped with Amicon® YMT membranes (Amicon, Danvers, Massachusetts, USA), that had been washed three times with bidistilled water. The total volume of ultrafiltrate obtained was approximately 2.2 ml. Separate experiments showed that during the procedure (from adjustment of the plasma pH until completion of ultrafiltration) the plasma pH does not change more than 0.05 units. After ultrafiltration 2 ml of the ultrafiltrate was alkalinised with 50 µl 1 N NaOH and extracted with 6 ml n-hexane for 1 min on a whirl mixer. Further preparation and analysis were as described elsewhere [10, 11]. The reproducibility of the method for determination of the degree of protein binding was examined in separate experiments. Coefficients of variation were 9% for R(+)-bupivacaine and 12% for S(−)-bupivacaine.

Concentrations of R(+)- and S(−)-bupivacaine in plasma and ultrafiltrate were measured by an enantioselective h.p.l.c. method [10] following a solid phase extraction procedure [11]. Coefficients of variation were 6.0, 5.0, and 2.9% for R(+)-bupivacaine and 10.4, 4.9 and 2.9% for S(−)-bupivacaine at plasma concentrations of 50, 250 and 500 ng ml^{-1}, respectively. The limits of determination (signal/noise ratio = 5) were 8 ng ml^{-1} R(+)-bupivacaine and 10 ng ml^{-1} S(−)-bupivacaine [11]. Coefficients of variation at these limits were 12.4% and 15%, respectively.

Pharmacokinetic and statistical analysis

The areas under the plasma drug concentration-time curves (AUC) and the first moment of these curves (AUMC) were estimated using the log-linear trapezoidal rule with addition of the area from the last sampling time until infinity [12]. Terminal rate constants (λ_Z) were determined by linear regression analysis of the latter part of the log plasma concentration-time curve of the individual enantiomers, generally from 180 or 240 min on. The values of the reported pharmacokinetic parameters were then derived from AUC, AUMC and λ_Z [12–14]. Plasma clearances and volumes of distribution based on unbound drug concentrations were determined by dividing the values based on total plasma drug concentrations by the free fraction measured in the blank sample.

Data are summarized as mean ± s.d. Pharmacokinetic data of R(+)- and S(−)-bupivacaine were compared with the paired *t*-test. In addition, 95% confidence intervals for the mean differences in the values of the pharmacokinetic parameters of the enantiomers were determined. Blood pressure data were subjected to repeated measures ANOVA, followed by the Dunnett test, if appropriate. $P < 0.05$ was regarded as statistically significant.

Results

The intravenous administration of rac-bupivacaine was well tolerated by all of the subjects and did not result in signs or symptoms indicative of systemic toxicity. Cardiovascular parameters did not change over time except for the systolic blood pressure, which decreased from a control value of 128 to 117 mm Hg after 45 min ($P < 0.01$). This decrease was probably not related to cardiac effects of bupivacaine,

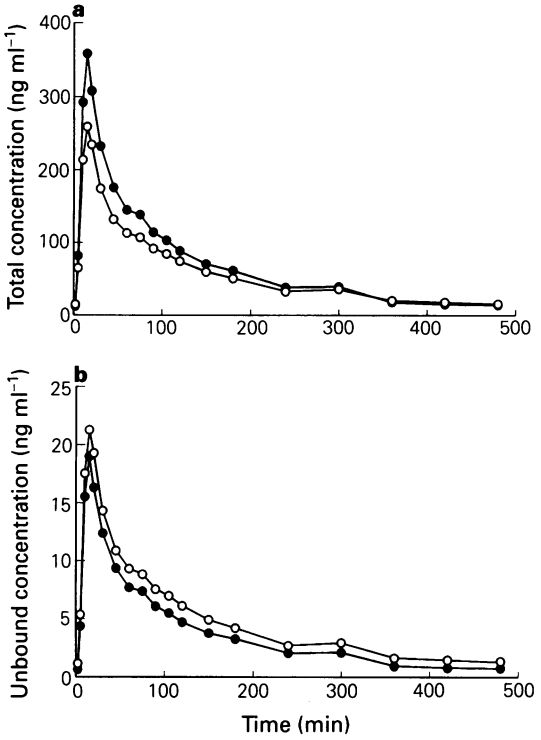

Figure 1 Total (a) and unbound (b) plasma concentrations of R(+)-bupivacaine (○) and S(−)-bupivacaine (●) in volunteer 3.

but may reflect an elevated control blood pressure, due to stress before the experiments began.

Plasma concentrations of the individual enantiomers in one of the subjects are shown in Figure 1. Although rac-bupivacaine was given by a 10 min intravenous infusion, the highest venous plasma concentrations of both R(+)- and S(−)-bupivacaine were generally reached at 15 min. In general, total plasma concentrations of S(−)-bupivacaine were higher than those of R(+)-bupivacaine. In five subjects the curves crossed between 360 and 420 min. However, unbound plasma drug concentrations, calculated by multiplying the total concentrations by the free fraction determined from the blank sample, were usually lower for S(−)-bupivacaine than for R(+)-bupivacaine (Figure 1).

Individual values of the fraction unbound (*fu*), plasma clearance (CL), volume of distribution at steady state (V_{ss}) and terminal half-life ($t_{1/2,z}$) are presented in Table 1. Mean values ± s.d. of these and other derived parameters and the statistical comparisons are presented in Table 2. Free fractions of R(+)-bupivacaine were higher than those of S(−)-bupivacaine in all subjects. CL, V_{ss} and the volume of distribution during the terminal log-linear phase (V), based on total plasma concentration, as well as $t_{1/2,z}$ and the mean residence time (MRT) of R(+)-bupivacaine were significantly greater than those of S(−)-bupivacaine. However, the plasma clearance (CLu) of

Table 1 Individual pharmacokinetic data of R(+)- and S(−)-bupivacaine

Subject	*fu(R)* (%)	*fu(S)* (%)	*CL(R)* (*l min*$^{-1}$)	*CL(S)* (*l min*$^{-1}$)	V_{ss}*(R)* (*l*)	V_{ss}*(S)* (*l*)	$t_{1/2,z}$*(R)* (*min*)	$t_{1/2,z}$*(S)* (*min*)
1	3.8	2.6	0.521	0.426	126	93	241	205
2	2.9	1.8	0.293	0.244	58	36	229	154
3	8.2	5.3	0.464	0.410	100	66	191	140
4	8.1	5.5	0.309	0.257	105	78	387	351
5	6.7	4.4	0.360	0.262	61	43	137	121
6	8.9	5.7	0.409	0.326	52	39	127	100
7	11.2	7.7	0.445	0.325	56	37	99	81
8	2.9	2.2	0.352	0.253	59	37	137	105
9	9.6	6.8	0.462	0.374	108	65	207	150
10	4.0	2.5	0.337	0.296	111	49	346	159

Table 2 Mean pharmacokinetic data (± standard deviation) of R(+)- and S(−)-bupivacaine

	R(+)-bupivacaine	*S(−)-bupivacaine*	*Ratio R(+)-/S(−)-bupivacaine*	P*	*95% confidence interval*‡
*f*u (%)	6.6 ± 3.0	4.5 ± 2.1	1.50 ± 0.09	< 0.0002	1.5 to 2.9
CL (l min^{-1})	0.395 ± 0.076	0.317 ± 0.067	1.25 ± 0.10	< 0.0001	0.059 to 0.097
CLu (l min^{-1})	7.26 ± 3.60	8.71 ± 4.27	0.84 ± 0.11	< 0.01	−2.44 to −0.46
V_{ss} (l)	84 ± 29	54 ± 20	1.56 ± 0.27	< 0.0002	19 to 40
Vu_{ss} (l)	1576 ± 934	1498 ± 892	1.04 ± 0.17	N.S.	−142 to 298
V (l)	117 ± 47	71 ± 34	1.70 ± 0.31	< 0.0002	29 to 61
*V*u (l)	2233 ± 1442	1978 ± 1302	1.13 ± 0.20	N.S.	−102 to 614
$t_{1/2,z}$ (min)	210 ± 95	157 ± 77	1.36 ± 0.31	< 0.01	18 to 89
MRT (min)	215 ± 74	172 ± 55	1.26 ± 0.28	< 0.02	10 to 76

*Paired *t*-test probability for the difference between R(+)- and S(−)-bupivacaine.
‡95% confidence interval for the mean difference between R(+)- and S(−)-bupivacaine.

unbound R(+)-bupivacaine was significantly lower than that of S(−)-bupivacaine. Volumes of distribution based on unbound drug concentrations (Vu_{ss} and Vu) did not differ between R(+)- and S(−)-bupivacaine.

Discussion

Whereas bupivacaine is used clinically as the racemate, there is increasing evidence that the enantiomers differ in their nerve blocking activity, toxicity and pharmacokinetics. *In vitro* studies, conducted more than 20 years ago, suggested that R(+)-bupivacaine and S(−)-bupivacaine are equipotent in producing nerve blockade [1]. However, a recent *in vitro* study indicated that R(+)-bupivacaine is more potent than S(−)-bupivacaine in inhibiting normal sodium channels [2]. *In vivo* studies in animals and man indicated that subcutaneous administration of S(−)-bupivacaine results in a longer duration of nerve block [1, 3]. This probably reflects a slower systemic absorption of S(−)-bupivacaine, owing to its greater vasoconstrictive activity [3]. The LD_{50} of S(−)-bupivacaine was found to be higher than that of R(+)-bupivacaine in various animal species [1]. In addition, recent studies in rats and in isolated guinea-pig preparations demonstrated a greater cardiotoxic potential of R(+)-bupivacaine [4–5]. Furthermore, a study in sheep indicated a greater central nervous system toxicity of R(+)-bupivacaine [6]. The observations suggest a difference in the intrinsic toxicity of the enantiomers, favouring the safety of S(−)-bupivacaine.

Recent studies of the pharmacokinetics of the enantiomers of bupivacaine, administered either separately or as the racemate in sheep, showed that the total and hepatic clearance of R(+)-bupivacaine were higher than those of S(−)-bupivacaine, whereas the mean total clearances of unbound drug appeared to be similar for both enantiomers [6–7]. Volumes of distribution, based on either total blood concentrations or unbound concentrations also appeared to be similar for R(+)- and S(−)-bupivacaine. No differences were found in the degree of plasma binding of the enantiomers [6]. However, in another study from the same group the plasma binding of S(−)-bupivacaine in sheep was found to be lower than that of R(+)-bupivacaine at higher plasma concentrations [15]. This observation in sheep contrasts with our findings in humans, suggesting that the enantioselectivity in the protein binding of bupivacaine is either dependent on the species or on the plasma concentration.

The present study demonstrated a higher plasma clearance of R(+)-bupivacaine, but the unbound clearance of R(+)-bupivacaine was found to be lower than that of S(−)-bupivacaine. The volumes of distribution of R(+)-bupivacaine, based on total plasma concentrations, were found to be larger than those of S(−)-bupivacaine, whereas the volumes of distribution, based on unbound plasma drug concentrations were found to be similar for both enantiomers. These observations indicate that the differences in the pharmacokinetics of the enantiomers of bupivacaine can largely be attributed to differences in their degree of plasma binding.

Although rac-bupivacaine was infused over 10 min, peak plasma concentrations of the enantiomers were not reached until 5–10 min after termination of the infusion. The delayed peak concentrations are probably a consequence of sampling from a peripheral vein and most likely reflect uptake of drug in the arm. Initial distribution characteristics, derived from venous concentrations, may differ somewhat from those, derived from arterial concentrations. Initial distribution volumes were not derived in this study, but inspection of the raw data indicates that the initial volume of distribution based on total plasma concentrations is greater for R(+)-bupivacaine than for S(−)-bupivacaine, whereas the initial volume of distribution based on unbound concentrations is likely to be similar for both enantiomers. Uptake in the arm should have minimal effect on the AUC, AUMC, or any parameters, derived thereof.

The plasma concentration ratios of R(+)- and S(−)-bupivacaine measured after interpleural injection of rac-bupivacaine followed by interpleural infusion of rac-bupivacaine for 24 or 48 h [Groen *et al.*, unpublished observations], were comparable with those observed in the present study. Therefore, differences in the degree of plasma binding may also explain most of the differences in the plasma concentrations of R(+)-bupivacaine and S(−)-bupivacaine following interpleural administration. Any contribution of an enantioselective systemic absorption rate remains to be investigated.

In conclusion, this study demonstrated that the systemic diposition of bupivacaine is enantioselective. Differences in the pharmacokinetics of R(+)- and S(−)-bupivacaine can largely be explained on the basis of enantioselective plasma binding. These data, along with the available data on the toxicity of the enantiomers provide a rational basis for development of stereochemically pure S(−)-bupivacaine as a local anaesthetic.

Racemic bupivacaine dry substance was donated by Astra Pharmaceutica (Rijswijk, The Netherlands). The authors thank Mrs M. C. O. van den Nieuwenhuyzen for her assistance with the experiments and Mrs E. A. Dullaart for her secretarial assistance.

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(Received 16 November 1993, accepted 11 April 1994)