PLASMA CONCENTRATIONS OF THE STEROISOMERS OF PRILOCAINE AFTER ADMINISTRATION OF THE RACEMATE: IMPLICATIONS FOR TOXICITY?†

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SUMMARY
A chiral high pressure liquid chromatography method was developed to measure the separate isomers of prilocaine in plasma after administration of the racemate. The concentrations of the isomers in six patients were similar (s(+) / R(—) = 1.06 (SD 0.06)) after brachial plexus block with 1.5% (rs)-prilocaine hydrochloride 35 ml, suggesting that a higher systemic safety margin may not be achieved by substituting racemic prilocaine by one of its isomers. Much higher plasma concentrations of the s(+) - than the R(—)-form after oral administration of 300 mg of the racemate (n = 4) indicated a large difference in intrinsic metabolic clearance of the isomers on first pass through gut, liver or both organs.

KEY WORDS
Anaesthetics, local: prilocaine. Toxicity: local anaesthetics.

(rs)-Prilocaine (α-propylamino-2-methylpropionanilide) is considered to be the local anaesthetic of choice for i.v. regional anaesthesia and brachial plexus block, as it has a relatively low potential for systemic CNS toxicity because of rapid blood clearance (2.4 litre min⁻¹) by both hepatic and extrahepatic metabolism [1, 2]. However, its use is limited essentially to single injection procedures because metabolism by amide hydrolysis and hydroxylation of the toluidine product is associated with methaemoglobinaemia [3]. Unlike lignocaine, which does not exhibit optical isomerism, but in common with other clinically used amide-type agents, prilocaine is given as a racemate (a 50:50 mixture of both isomers). Therefore, as the ratio of the intrinsic anaesthetic activities of the enantiomers is small [4], the possibility of stereoselective metabolism might suggest a further advantage with respect to systemic safety in using a single isomer. To investigate this, a method was developed to measure the separate plasma concentrations of s(+) - and R(—)-prilocaine by chiral column high pressure liquid chromatography (HPLC) after administration of the racemate.

PATIENTS AND METHODS
Brachial plexus block was performed on six surgical patients using (rs)-prilocaine hydrochloride 525 mg (35 ml of 1.5% solution). These patients formed part of a larger study of the influence of different techniques of brachial plexus block on the systemic absorption of prilocaine, which was described in detail by Maclean and colleagues [5]. In addition, four healthy male anaesthetists received (rs)-prilocaine hydrochloride 300 mg by mouth as an aqueous solution. Both studies were approved by the local Ethics Committee. The latter study was included to assess the relative intrinsic metabolism of the isomers uncomplicated by the influence of blood flow on clearance. Serial venous blood samples were taken up to 60 min after brachial plexus injection and up to 120 min after the oral dose. Plasma concentrations of unresolved drug were measured by gas–liquid chromatography (GLC). Alkalinized plasma 1.0 ml containing internal standard (ethyldimethylglycinexylylidide)
was extracted with diethylether 5 ml, followed by back-extraction into 200 μl of hydrochloric acid 1 mol litre⁻¹. This was then alkalinized and extracted with cyclohexane 40 μl, 5-10 μl of which was injected into the gas chromatograph. An OV-225 column (2 m x 4 mm i.d.) was used together with a nitrogen detector. The lower limit of this method was 50 ng ml⁻¹, at which concentration the intra-assay coefficient of variation was 7% (n = 10).

The ratio of the isomers was estimated using a 4.6-mm i.d. x 250-mm HPLC column containing cellulose tris-3,5-dimethylphenyl carbamate coated on silica gel (Chiralcel OD; Daicel Chemical Industries Ltd), after extracting the compounds from alkalinized plasma 0.5 ml into n-hexane 300 μl. The column was eluted with n-hexane:3-propanol:diethylamine (80:20:0.01) at a flow rate of 1 ml min⁻¹, and u.v. detection was at 263 nm. Powdered (RS)-prilocaine hydrochloride and the commercial solution for injection were used as standards to assess the relative concentrations of the isomers in plasma samples. Thus isomer ratios in the latter were compared with those found after extraction of blank plasma spiked with comparable concentrations of the standards. The isomer ratio was measured with a precision of ±4% at concentrations of individual isomers down to 50 ng ml⁻¹. It was not possible to assay the concentrations of each isomer directly using the chiral column because a suitable internal standard was not found.

Maximum values of plasma drug concentrations (Cmax) were noted and areas under the plasma drug concentration–time curves (AUC) to the last datum point were estimated using the linear trapezoidal rule. Differences between the values observed for the two isomers were evaluated using Student’s paired t test, assuming significance at P < 0.05.

RESULTS

Complete baseline separation of the isomers was achieved using the chiral column, with retention times of 6.8 min for (S)- and 8.2 min for (R)-prilocaine (fig. 1). The column proved to be robust, although the guard-column containing the same material had to be replaced after 500 injections. Prilocaine was stable in frozen plasma and there was no evidence of a change in isomer ratios or inversion of the isomers on prolonged storage. Isomer ratios in samples spiked with the standards were independent of total racemic drug concentration over the working range, and there was no difference in the ratio as a function of the source of the standard (powder or solution for injection).

Mean plasma concentrations of the individual isomers after the two routes of administration of the racemate are shown in figure 2. Concentrations of the (R)- isomer after oral administration of (RS)-prilocaine were very low and below the limit of assay at most time points in all subjects. Mean (SD) values of AUC(0, 60 min) and Cmax following brachial plexus injection were 43 (15) μg ml⁻¹ min and 1023 (352) ng ml⁻¹ for (S)-prilocaine and 41 (17) μg ml⁻¹ min and 990 (381) ng ml⁻¹ for (R)-prilocaine. The differences between the isomers were statistically significant (paired test, P < 0.05). After oral administration AUC(0, 120 min) and Cmax values were 9.2 (2.4) μg ml⁻¹ min and 157 (81) ng ml⁻¹, respectively for (S)-prilocaine, but could not be estimated accurately for the (R)-isomer. The average (S): (R) ratio for all samples in each patient after brachial plexus injection was 1.06 (0.06), with no evidence of a change with time. In contrast, after oral administration plasma

![Fig. 1. Chiral high pressure liquid chromatograms of extracts from A: blank plasma; B: plasma from a patient after injection of (RS)-prilocaine for brachial plexus block (S+) 1.59 μg ml⁻¹; R(−) 1.15 μg ml⁻¹). 0.01 absorbance units, full scale deflection.](image-url)
concentrations of S(+)- drug were at least eight times greater than those of the R(−)-isomer. Values of the time to Cmax were in the range 15–60 min following both routes of drug administration.

**DISCUSSION**

The marked route-dependence of the plasma s:R ratio may be explained by blood flow-limited systemic clearance of both isomers after parenteral injection; and an appreciable first-pass effect after oral administration manifesting a large difference in the intrinsic metabolic clearance of the antipodes. Thus theory predicts that the kinetics of a drug with a high hepatic clearance should be blood flow-dependent after parenteral administration, but dependent largely upon enzyme activity after oral administration [6]. In addition, stereoselective presystemic metabolism within the gut wall might contribute to the large difference in plasma concentrations of the isomers after oral administration. Any difference in the plasma binding of the isomers would contribute also to the differences seen in their total plasma concentrations. However, such a difference would not be route-dependent and the extent of binding of prilocaine is relatively low [7]. Assuming that absorption is a passive process, a difference in the relative absorption rate of the isomers from the site of administration cannot account for the observations, as enantiomers have identical physico-chemical properties. Furthermore, any stereoselectivity of their effects on local blood flow would not influence relative absorption rate when the isomers are given together as a racemate.

As the isomers of prilocaine show little difference from the racemate in their anaesthetic effect and in their i.v. LD50 values in animals [4,8], and we have shown that their plasma concentrations after perineural injection of the racemate are similar in man, this supports the view that a higher safety margin for systemic CNS toxicity may not be achieved by substituting racemic prilocaine by one of its isomers. A caveat to this is that a slower systemic uptake of one isomer, as a result of differential effects on local blood flow, could recommend it over the racemate. Such differences have been observed in animals [4], but they appear to be relatively minor.

A further issue is that, if significantly smaller plasma concentrations of one isomer had been shown following perineural injection, this would have to be set against the possibility of more rapid formation of the metabolic products responsible for methaemoglobinemia. Thus Akerman and Ross [4] observed smaller plasma concentrations of R(−)-prilocaine after i.v. injection of the separate isomers into cats, but the rate of production of methaemoglobin was correspond-
ingly faster. It should be noted that several authors have misrepresented this work in proposing prilocaine as an example of the clinical use of a single isomer being preferred over the racemate. Thus it has been claimed that only the R(—)-isomer undergoes metabolic hydrolysis to toluidine and hence produces methaemoglobinemia [9–11]. The original work notes merely a difference in the rate and not the extent of methaemoglobin formation after administration of the isomers [4].

The abolition of the centre of asymmetry in prilocaine by addition of another methyl group, to produce quatacaine, is claimed to obviate the problem of methaemoglobinaemia while retaining the anaesthetic profile [11, 12]. However, this change is at the expense of increased CNS toxicity, probably as a result of protecting the amide linkage against rapid hydrolysis. It remains difficult to see how the structure of prilocaine might be modified to lower the propensity for methaemoglobin formation, while retaining the high systemic clearance and safety margin with respect to CNS toxicity.

ACKNOWLEDGEMENTS

We thank Astra Pain Control AB, Södertälje, Sweden and The Swan–Morton Foundation for their contributions towards the cost of the chiral HPLC column. Samples of the isomers of prilocaine were kindly supplied by Dr B. Akerman (Astra AB).

REFERENCES