ABSTRACT:
The objective of this study was to compare the blood-brain barrier (BBB) transport and brain distribution of levo- (R-CZE) and dextro-cetirizine (S-CZE). Microdialysis probes, calibrated using retrodialysis by drug, were placed into the frontal cortex and right jugular vein of eight guinea pigs. Racemic CZE (2.7 mg/kg) was administered as a 60-min i.v. infusion. Unbound and total concentrations of the enantiomers were measured in blood and brain with liquid chromatography-tandem mass spectrometry. The brain distribution of the CZE enantiomers were compared using the parameters $K_{p}$, $K_{p,u}$, $K_{p,uu}$, and $V_{u,br}$. $K_{p}$ compares total brain concentration to total plasma concentration, $K_{p,u}$ compensates for binding in plasma, whereas $K_{p,uu}$ also compensates for binding within the brain tissue and directly quantifies the transport across the BBB. $V_{u,br}$ describes binding within the brain. The stereoselective brain distribution indicated by the $K_{p}$ of 0.22 and 0.04 for S- and R-CZE, respectively, was caused by different binding to plasma proteins. The transport of the CZE enantiomers across the BBB was not stereoselective, since the $K_{p,uu}$ was 0.17 and 0.14 (N.S.) for S- and R-CZE, respectively. The $K_{p,uu}$ values show that the enantiomers are effluxed to a large extent across the BBB. The $V_{u,br}$ of approximately 2.5 ml/g brain was also similar for both the enantiomers, and the value indicates high binding to brain tissue. Thus, when determining stereoselectivity in brain distribution, it is important to study all factors governing this distribution, binding in blood and brain, and the BBB equilibrium.

Distribution of drugs to the brain is restricted by the presence of the blood-brain barrier (BBB). Tight junctions between the endothelial cells of the brain capillaries form the BBB and act as a self-defense mechanism, preventing xenobiotics from entering the brain. Successful penetration of the BBB is necessary for drugs to have a central nervous effect. However, reduced penetration of some drugs enables their side effects to be avoided. Cetirizine (CZE), an H1-receptor antagonist, is an example of such a drug. CZE is a non-sedating second-generation antihistamine and is widely prescribed for seasonal and perennial allergic rhinitis and chronic idiopathic urticaria. It has been shown both in animal models and humans that cetirizine enters the brain to a low extent and occupies not more than 30% of the human H1-receptor compared with racemic CZE and approximately 30-fold higher affinity than dextrocetirizine (S-CZE). The difference in affinities between the two enantiomers is mainly accounted for by their different dissociation rates from H1-receptors, with R-CZE demonstrating a far longer dissociation half-life (142 min) than S-CZE (6 min) (Gillard et al., 2002). The pharmacokinetics of CZE in blood is known to be stereoselective in humans (Baltes et al., 2001). However, studies comparing the brain distribution of CZE enantiomers are lacking. The difference in the penetration of the enantiomers could result in different availability of the enantiomers for the H1-receptor in the brain.

The distribution of drugs to the brain is determined by various factors including the transport across the BBB and their binding in blood and in the brain. Differences in enantiomer binding in blood and/or brain could cause stereoselective brain distribution. Also, if active influx and/or efflux transporters at the BBB are involved, the transport per se could be stereoselective. Hence it is important to investigate which of the above factors, if any, play a major role in stereoselective brain distribution of the CZE enantiomers.

The microdialysis technique is being increasingly applied in pharmacokinetic studies to characterize drug transport to the brain in vivo (Elmqist and Sawchuk, 1997; de Lange et al., 1999; Hammarlund-Udenaes, 2000). By placing the probes in both the blood and brain it is possible to measure unbound concentrations on each side of the BBB in one animal over time and hence characterize the transport across the BBB. Measurement of total concentrations in plasma and brain were presented at Pharmaceutical Sciences World Congress 2004, Kyoto, Japan, and UCB Société Anonyme, Braine-l’Alleud, Belgium (P.C., R.M.).

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ABBREVIATIONS: BBB, blood-brain barrier; CZE, cetirizine; R-CZE, levocetirizine; S-CZE, dextrocetirizine; ISF, interstitial fluid; IS, internal standard; BSA, bovine serum albumin; AUC, area under the curve; CL, clearance.

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BRAIN DISTRIBUTION OF CETIRIZINE ENANTIOMERS: COMPARISON OF THREE DIFFERENT TISSUE-TO-PLASMA PARTITION COEFFICIENTS: $K_{p}$, $K_{p,u}$, AND $K_{p,uu}$

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brain along with unbound concentrations will give a measure of binding in blood and brain, respectively. Knowing that 1) R-CZE is the active enantiomer, 2) sedation is correlated with the amount of drug entering into the brain, and 3) an active efflux process is involved in the transport of CZE across the BBB, the aim of this work was to investigate the brain distribution of CZE enantiomers in guinea pigs after administration of the racemic CZE, to exclusively measure and quantify the BBB transport, and also to characterize the processes involved in stereoselectivity. Information on brain distribution and transport across the BBB of CZE will be helpful in better understanding the mechanisms involved in low sedation of this second-generation antihistamine. Characterization of the processes involved in stereoselective brain distribution of CZE will help in differentiating the effect of binding, in blood and brain, from the transport across the BBB.

Materials and Methods

Probes and Chemicals. Racemic CZE, the pure enantiomers S- and R-CZE, and an internal standard (IS, ucb20028), (2-[2-(4-benzhydrolydine-piperidin-1-yl)-ethoxy]-ethoxy}-acetic acid chlorhydrate, were supplied by UCB Pharma (Braine l’Alleud, Belgium). Isoflurane, saline, Ringer (33 mg of KCl, 330 mg of CaCl₂·H₂O, 8.6 g of NaCl, sterilized water for injection, Ph. Eur. Ad 1000 ml, pH 6) was purchased from Hospital Pharmacy (Uppsala, Sweden). Low molecular weight heparin and bovine serum albumin (BSA) (initial fractionation by heat shock) were purchased from Sigma Chemical Co. (St Louis, MO). All chemicals were of analytical grade and solvents were of high-performance liquid chromatography grade. The water was purified by a Milli-Q Academic system (Millipore, Bedford, MA). Microdialysis probes, CMA/12 (3 mm and 4 mm) and CMA/20 (10 mm), were purchased from CMA Microdialysis (Solna, Sweden). The probe membranes were made of polycarbonate and have a molecular mass cutoff of 20 kDa.

Animals. The guinea pig was selected as the animal model since it has high brain concentrations of H₁-receptors compared with rats and is often used in antihistamine work (Hill and Young, 1980). Male Dunkin Hartley guinea pigs (n = 8) weighing 350 to 550 g (Mollegaard, Skensved, Denmark and Charles River Laboratories, L’Arbresles, France) were acclimatized for at least 5 days at 22°C and controlled humidity before the experiment. Standard diet and water were provided ad libitum. The protocol was approved by the Animal Ethics Committee of Uppsala University (C218/1).

Probe Characterization in Vitro. Microdialysis probes were calibrated using the retrodialysis by drug method (Bouw and Hammarlund-Udenaes, 1998), which assumes that the recovery is the same in both directions across the BBB. This was verified in vitro before performing in vivo experiments. For direct dialysis (gain of analyte from external medium to probe), the probe (brain probe, CMA/12 3 mm) was placed in an Eppendorf tube containing blank Ringer solution for 30 min, followed by Ringer solution containing CZE (50 ng/ml, Cₐ) for 90 min. The probe was then placed back in the blank Ringer solution for 120 min for washout. The perfusion fluid was blank Ringer solution during the direct dialysis experiment. For retrodialysis (loss of analyte from probe to external medium), after washout, the same probe was perfused with Ringer solution containing CZE (50 ng/ml, Cₐ) for 90 min, followed by blank Ringer solution for 120 min. The probe was placed in blank Ringer solution during the retrodialysis experiment. The external medium was stirred constantly. The experiments were performed at 37°C in triplicate. The perfusion fluids investigated were Ringer solution and Ringer solution with 0.5% BSA (w/v). The dialysate fractions were collected every 15 min (Cₐ) and the flow rate was 0.5 μl/min. The relative recovery of CZE enantiomers in vitro by the direct dialysis method was calculated by the formula, Recovery = C₀ₐ/Cₐ. The recovery and retrodialysis recovery was determined by the formula, Recovery = (C₀ₐ-Cₐ)/C₀ₐ.

Surgical Procedure. The guinea pigs were anesthetized by inhalation of isoflurane (2.5% balanced with 1.5 l/min oxygen and 1.5 l/min nitrous oxide) and 0.25 ml of Dormicum (midazolam 5 mg/ml) intraperitoneally. The guinea pigs were placed on a heating pad to maintain the body temperature at 38°C during surgery. FEP tubing was inserted into the left jugular vein for drug administration and into the left common carotid artery for blood sampling. The catheters were filled with heparinized saline solution (100 IU/ml) to prevent clotting. The blood probe (CMA/20, polycarbonate, 20 kDa cutoff, 10 mm) was inserted into the right jugular vein through a guide cannula and fixed in the pectoral muscle with two sutures. For inserting the brain probe, the guinea pig was placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA), and a midline incision was made to expose the skull. A hole was drilled into the skull at ~1.0 mm lateral and 1.1 mm anterior to the bregma point. At these coordinates and 3.2 mm ventral to the surface of the brain, a CMA/12 guide cannula with a dummy probe was implanted into the frontal cortex and fixed to the skull with a screw and dental cement. When the cement had set, the dummy probe was replaced with a 3-mm CMA/12 brain probe.

The ends of the catheters were passed subcutaneously to a plastic tube placed on the surface of the neck, out of reach of the guinea pig. The guinea pig was placed in a CMA/120 system for freely moving animals, with free access to water and food, and the experiment was performed approximately 24 h later.

Probe Calibration in Vivo. Nonbuffered Ringer solution containing 0.5% BSA was used as the perfusate in the study. To stabilize the system and to obtain blank samples for the chemical analysis, the probes were perfused with a blank Ringer with BSA at a flow rate of 0.5 μl/min, by means of a CMA/100 microinjection pumps (CMA Microdialysis). Samples were collected at 15-min intervals for 60 min. All the probes were calibrated in vivo according to the retrodialysis by drug method (Bouw and Hammarlund-Udenaes, 1998). During the retrodialysis period, the perfusion solution for the blood probe and the brain probe contained 50 and 25 ng/ml CZE in Ringer with BSA, respectively. In total, five samples, in fractions of 15 min, were collected during this period. The perfusion fluid was subsequently changed to blank Ringer with BSA. A washout period of 90 min was allowed before systemic drug administration. The relative recovery of CZE was estimated in each guinea pig, and a mean value of at least three estimations during the retrodialysis period was used to calculate the unbound concentrations of S- and R-CZE in brain interstitial fluid (ISF) and blood from the dialysate concentrations.

Study Design. CZE was administered as a 60-min constant rate infusion, resulting in a total dose of 2.7 ng/kg. The maximum total plasma target concentration was 3000 ng/ml. Blood and brain ISF was continuously sampled by microdialysis. During the infusion and 1 h postinfusion, dialysates were collected every 15 min, and then every 30 min for the next 4 h. Arterial blood samples were collected at 0, 5, 10, 30, 60, 90, 120, 180, 240, and 360 min, and the plasma was separated by centrifugation for 5 min at 10,000 rpm. The guinea pig was decapitated at the end of the experiment, and the brain was weighed and saved for analysis of the CZE enantiomers. All the samples were stored at −20°C until analysis.

Chemical Analysis. Guinea pig plasma, brain, and microdialysis blood and brain ISF samples were analyzed using a liquid chromatography-tandem mass spectrometry method described previously (Gupta et al., 2005). In brief, 50 μl of plasma was precipitated with 100 μl of acetonitrile containing IS. A portion of the supernatant (50 μl) was evaporated, the residue was dissolved in 500 μl of the mobile phase, and 50 μl was injected into the liquid chromatography-tandem mass spectrometry system. The chromatographic separation of the CZE enantiomers was achieved by a Chiral-AGP (α-acid glycoprotein) column (α-acid glycoprotein, 150 × 4.0 mm; ChromTech, Hägersten, Sweden) in tandem with a triple quadrupole mass spectrometer (Quattro Ultima; Waters, Manchester, UK). The mobile phase consisted of 10 mM ammonium acetate, pH 7.0 (adjusted with 1% ammonia), and 6.5% acetonitrile. The assay was linear over the range of 0.25 (CV 13%) to 5000 ng/ml for both S-CZE and R-CZE. To analyze the brain samples, each brain was homogenized with a 4-fold volume of saline, and the homogenate was processed in a manner similar to that described for plasma samples. The assay was linear over the range 2.5 (CV 10%) to 250 ng/g brain for both S-CZE and R-CZE. The BSA in the microdialysis samples was precipitated using acetonitrile with IS before injecting onto the column. The assay was linear over the range of 0.25 (CV 13%) to 250 ng/ml for both the enantiomers.

Basic Relationships. To characterize brain distribution of the CZE enantiomers, three different partition coefficients, Kₚ, Kₚ,n, and Kₚ,m,n were calculated. The partition coefficient Kₚ was calculated as...
where $AUC_{tot,br,0-\infty}$ and $AUC_{tot,pl,0-\infty}$ are the areas under the curve of total concentrations versus time in brain and plasma, respectively. The $K_p$ can also be expressed as

$$K_p = \frac{AUC_{tot,br,0-\infty}}{AUC_{tot,pl,0-\infty}}$$

where $AUC_{u,brISF,0-\infty}$ and $AUC_{u,pl,0-\infty}$ are the areas under the curve of unbound concentrations versus time in brain ISF and plasma, respectively. The $f_{us}$ is the unbound fraction of the drug in plasma and $f_{u,brISF}$ is the ratio of unbound concentrations in brain ISF to the total amount per gram of brain tissue. Thus, the $K_p$ value is a composite of the BBB equilibrium and the tissue and protein bindings in brain and blood.

To compensate for differences in plasma protein binding, the partition coefficient $K_{pu}$, was calculated as:

$$K_{pu} = \frac{AUC_{u,brISF,0-\infty}}{AUC_{u,pl,0-\infty}}$$

or, expressed differently,

$$K_{pu} = \frac{AUC_{u,brISF,0-\infty} \cdot AUC_{u,pl,0-\infty}}{f_{u,brISF} \cdot AUC_{pl,0-\infty}}$$

Thus $K_{pu}$ is a composite of the BBB equilibration and binding within the brain.

The ratio of the area under the unbound brain ISF concentration-time profile and area under the unbound plasma concentration-time profile was calculated to compensate for the binding also within the brain tissue. This ratio, in analogy to the partition coefficients above, is called the unbound partition coefficient, $K_{pu,un}$ and directly describes the equilibrium across the BBB.

$$K_{pu,un} = \frac{AUC_{u,brISF,0-\infty}}{AUC_{u,pl,0-\infty}}$$

Binding within the brain can be estimated using $f_{u,brISF}$. However, the magnitude of $f_{u,brISF}$ is proportional, but not necessarily equal, to the unbound fraction in brain ISF. The true unbound fraction in brain ISF will be $\delta \cdot f_{u,brISF}$, where $\delta$ is the fraction of brain volume in which the unbound drug is distributed (Fichtl, 1991). This fraction could be anything between ISF volume and total brain water volume. A more intuitive way of estimating binding within the brain is using the unbound volume of distribution in brain ($V_{u,br}$) expressed as

$$V_{u,br} = \frac{(A_u - V_t \cdot C_t)}{C_{u,brISF}}$$

where $A_u$ is the total amount of the CZE enantiomers per gram of brain, $V_t$ is the volume of blood per gram of brain, $C_t$ is the total concentration in blood, and $C_{u,brISF}$ is the unbound concentration in brain ISF (Wang and Welty, 1996). Thus $V_{u,br}$ can also be expressed as $1/f_{u,brISF}$. As $V_{u,br}$ has the free drug in brain ISF as the reference ($C_{u,brISF}$), the obtained values can be compared with physiological volumes. The brain extracellular space is reported as 0.12 to 0.20 ml/g brain (Levin et al., 1970; Goodman et al., 1973). Thus, a volume close to 0.2 ml/g brain shows that the drug is mainly distributed into brain ISF, and a higher value indicates that drug is distributed intracellularly and/or binds to protein in ISF.

For cetyltrimethylammonium, the total concentration-time profile in brain was generated using the unbound concentration-time profile in brain ISF obtained from the microdialysis experiment and the fraction unbound in brain ISF at 360 min after the infusion ($f_{u,brISF,360}$), with the assumption that the fraction unbound of the CZE enantiomers in brain was constant with time. To estimate binding of CZE enantiomers within the brain, the $V_{u,br}$ at 360 min was calculated. For the calculation of $V_{u,br}$ (eq. 6), plasma volume and total plasma concentrations were used, with the assumption that the CZE enantiomers are not or are only very poorly associated with blood cells in guinea pigs (Benedetti et al., 2001). The plasma content in the brain of the guinea pig has been estimated as 11.5 µl per gram of brain (Bosse and Wassermann, 1970). $A_u$ was determined in the whole brain sample taken at 360 min.

Individual plasma clearance (CL) and apparent volume of distribution (V) were computed as $CL = Dose_t/AUC_{u,pl,0-\infty}$ and $V = AUC_{u,pl,0-\infty} / CL_t$, respectively, where $\lambda$ is the terminal rate constant, obtained by log-linear regression of the terminal phase of the total concentration versus time curve. The unbound clearance (CL$_u$) and volume of distribution (V$_u$) in plasma were also calculated as described above, using the unbound concentration-time curve in plasma obtained from the microdialysis experiment. The terminal half-life in plasma ($t_{1/2,u}$) and brain ($t_{1/2,br}$) was expressed as ln2/CL$_u$ and ln2/CL$_t$, respectively. The $\lambda_u$ and $\lambda_t$ are the terminal rate constant obtained by log-linear regression of the terminal phase of the unbound concentration versus time curve in plasma and brain ISF, respectively. The fraction unbound in plasma ($f_{u,pl}$) was determined as the ratio of $AUC_{u,pl,0-\infty}$ and $AUC_{tot,pl,0-\infty}$.

The whole areas under the concentration-time curves, $AUC_{u,br}$, were expressed as the sum of area under the corresponding concentration versus time curve until the last observation (AUC$_{u,br}$,t) and the residual area (AUC$_{u,br}$,r). The AUC$_{u,br}$ was calculated by the trapezoidal method. The AUC$_{u,br}$ was determined as the ratio of the concentration at the last time point to respective terminal rate constants.

The two enantiomers were compared for brain distribution and blood pharmacokinetic parameters using exact Wilcoxon’s signed-rank test (S-plus 6.1 for Windows; Insightful Corp., Seattle, WA), because the sample number was too low to assume normal distribution of the population parameters. However, for the parameters $K_p$, $K_{pu}$, $K_{pu,un}$ and $f_{us}$ for S- and R-CZE were compared using a paired t test, since proportions are normally distributed. A p value of ≤0.05 was considered to be statistically significant.

Results

The in vitro characterization of the microdialysis probes showed that inclusion of 0.5% BSA in Ringer was necessary for the recovery of the CZE enantiomers to be equal in both directions across the probe membrane. It was also contributing to a more rapid equilibration across the probe membrane. The in vitro recoveries of S- and R-CZE determined by direct dialysis of the brain probes were 48.0 ± 4.59% and 47.8 ± 4.90%, and those by retrodialysis were 44.9 ± 5.10% and 44.4 ± 5.11%, respectively (N.S.). The in vivo recoveries of the CZE enantiomers with the brain probes were 18.5 ± 8.8% and 18.8 ± 8.4% for S- and R-CZE, respectively. With the blood probes, the in vivo recoveries were 83.5 ± 11.7% and 86 ± 10.5%, respectively.

The concentration-time profiles of the CZE enantiomers in plasma and brain ISF are shown in Fig. 1. The total concentrations of R-CZE in plasma were higher than those of S-CZE, whereas the unbound concentrations of R-CZE were lower than those of S-CZE. As in plasma, the unbound levels of R-CZE in brain ISF were lower than those of S-CZE. The AUC$_{u,pl,0-\infty}$, AUC$_{u,brISF,0-\infty}$, and $A_u$ were significantly lower for R-CZE than for S-CZE (Table 1).

FIG. 1. Total concentration-time profile in blood and unbound concentration-time profiles (average ± S.D.) in blood and brain ISF of R-CZE and S-CZE obtained from regular blood sampling and microdialysis, respectively. The data were collected during a 1-h constant rate infusion of racemic CZE to eight guinea pigs and 5 h after the end of infusion. All the unbound concentrations were corrected for recovery obtained by the retrodialysis by drug method before drug administration.
ever, the transport process consists of various transporter proteins involved in the active influx of drugs to and from the brain. Thus, the transport process is driven by a gradient between the brain and blood, which is established by the brain-blood barrier (BBB). The BBB is a dynamic barrier that separates the brain from the blood and is composed of the following components: the blood-brain barrier (BBB), the blood-cerebrospinal fluid (CSF) barrier, and the blood-tissue barrier. The BBB is formed by the endothelial cells of brain capillaries, which are connected by tight junctions and lack fenestrations. The BBB functions to regulate the passage of substances between the blood and the brain, and it is thought to be responsible for the selective permeability of the brain to various substances.

The BBB transport is also influenced by factors such as the plasma protein binding, the distribution of drugs in the brain, and the metabolic effects of drugs in the brain. The distribution of drugs in the brain is influenced by factors such as the pharmacokinetics of drugs in the brain, the blood-brain barrier transport, and the metabolic effects of drugs in the brain. The pharmacokinetics of drugs in the brain is influenced by factors such as the plasma protein binding, the distribution of drugs in the brain, and the metabolic effects of drugs in the brain. The plasma protein binding of drugs is influenced by factors such as the plasma protein binding, the distribution of drugs in the brain, and the metabolic effects of drugs in the brain. The distribution of drugs in the brain is influenced by factors such as the plasma protein binding, the distribution of drugs in the brain, and the metabolic effects of drugs in the brain. The metabolic effects of drugs in the brain are influenced by factors such as the plasma protein binding, the distribution of drugs in the brain, and the metabolic effects of drugs in the brain.

The brain distribution of CZE enantiomers determined by the $K_p$ can be interpreted as enantioselective, the value being 0.22 for S-CZE and 0.04 for R-CZE. Because $K_p$ compares total brain concentration to total plasma concentration, this measure includes all three of the factors governing the distribution: protein binding in blood, binding to brain parenchymal cells and proteins, and transport across the BBB (eq. 2). A higher $K_p$ for S-CZE indicates that one or several of these factors are different. It has been shown recently for 23 clinically used central nervous system drugs that the 50-fold range in absolute $K_p$ values was largely determined by binding in brain and blood (Maurer et al., 2005). The $K_p$ for racemic CZE in mice has previously been reported to be 0.02 and 0.05 (Chen et al., 2003; Polli et al., 2003).

The $K_{pu}$ accounts for the differences in binding to blood components (eq. 4). The plasma protein binding of CZE enantiomers was found to be stereoselective, with an fD of 0.50 and 0.15 for S- and R-CZE, respectively. However, the brain distribution of CZE enantiomers was found to be stereoselective, with an fD of 0.50 and 0.15 for S- and R-CZE, respectively. The $K_{pu}$ of 0.44 for S-CZE against 0.29 for R-CZE indicates that the stereoselectivity in brain partitioning observed with $K_p$ is mainly explained by the difference in plasma protein binding.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S-CZE</th>
<th>R-CZE</th>
<th>Ratio S/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (ml/min/kg)</td>
<td>7.29 ± 1.2</td>
<td>2.78 ± 0.62</td>
<td>2.6**</td>
</tr>
<tr>
<td>CLq (ml/min/kg)</td>
<td>14.9 ± 2.10</td>
<td>18.8 ± 3.67</td>
<td>0.8**</td>
</tr>
<tr>
<td>V (ml/kg)</td>
<td>1385 ± 277</td>
<td>593 ± 151</td>
<td>2.3**</td>
</tr>
<tr>
<td>Vd (ml/kg)</td>
<td>2808 ± 602</td>
<td>3028 ± 578</td>
<td>0.9</td>
</tr>
<tr>
<td>fD (h)</td>
<td>0.50 ± 0.10</td>
<td>0.15 ± 0.03</td>
<td>3.3***</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>2.20 ± 0.45</td>
<td>1.90 ± 0.45</td>
<td>1.2**</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (min · ng/ml)$^{d}$</td>
<td>68510 ± 9104</td>
<td>394 ± 1.63$^b$</td>
<td>1.3**</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (min · ng/ml)$^{d}$</td>
<td>11435 ± 2843</td>
<td>7498 ± 1728</td>
<td>1.5**</td>
</tr>
<tr>
<td>Apu (ng/g brain)$^d$</td>
<td>45.9 ± 13.3</td>
<td>25.4 ± 9.0</td>
<td>1.8**</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, and ***p < 0.001 for difference between the parameter for S-CZE and R-CZE.

Fig. 2. The three partition coefficients, $K_p$, $K_{pu}$, and $K_{pu,b}$ of S- and R-CZE (average ± S.D.; n = 8) measuring brain distribution of the enantiomers in guinea pigs. Both the $K_p$ and $K_{pu}$ for S-CZE were significantly higher than those for R-CZE (p < 0.001).

Fig. 3. Unbound volume of distribution, $V_{u,br}$ of S- and R-CZE in brain (average ± S.D.; n = 8). The $V_{u,br}$ is a measure of binding within the brain.
binding. However, factors other than plasma protein binding are likely to be involved. Transport across the BBB and/or binding in the brain could also be stereoselective. The $K_{p,u}$ for racemic CZE has previously been reported to be 0.23, measured until 4 h in mice (Polli et al., 2003). Comparison of $K_{p,u}$ for propranolol (Takahashi et al., 1990) and disopyramide (Hanada et al., 1998) enantiomers showed that the stereoselectivity observed in brain distribution of these drugs could be explained by stereoselective protein binding in blood.

Because $K_{p,u}$ is calculated from the unbound concentrations on both sides of the BBB (eq. 5), it most closely describes the transport across the BBB independent of binding to the brain (Hammarlund-Udenaes et al., 1997), and can therefore directly measure any stereoselectivity in active transport across the BBB. The $K_{p,u}$ was 0.17 and 0.14 for S- and R-CZE, respectively. A $K_{p,u} < 1$ indicates that active efflux processes are acting on CZE at the BBB (Hammarlund-Udenaes et al., 1997), and confirms and quantifies the involvement of P-glycoprotein at the BBB reported by Chen et al. (2003) and Polli et al. (2003). The similar $K_{p,u}$ values for S- and R-CZE indicate that the transport across the BBB is the same and that the active efflux at the BBB by P-glycoprotein does not differ between the two enantiomers. Our results fit with the results from Whomsley et al. (2003), who showed no difference in transport characteristics of racemic CZE and R-CZE in Caco-2 cell monolayers. S-CZE was not studied separately (Whomsley et al., 2003).

The binding of CZE enantiomers within the brain was measured with $V_{u,p}$. It compares the total brain concentration of a drug with its unbound ISF concentration and, therefore, describes the distribution within the brain irrespective of blood to brain distribution. The $V_{u,p}$ of 2.86 ml/g brain for S-CZE was not significantly different from the value of 2.39 ml/g brain for R-CZE, indicating no difference in the distribution and/or binding of CZE enantiomers within the brain. A significant difference observed for the $K_{p,u}$ could be explained by eq. 4. The $K_{p,u}$ is a product of $V_{u,p}$ $(f_{u,p}^{body})$ and $f_{p,u}^{brain}$. The $V_{u,p}$ and $K_{p,u}$ are not different for the two enantiomers; however, there is a trend for both being higher for S-CZE, leading to a significant difference in the $K_{p,u}$ value. For guinea pigs, if the ISF volume in the brain is assumed to be similar to that observed in other animal species (Levin et al., 1970; Goodman et al., 1973), a $V_{u,p}$ of approximately 2.5 ml/g brain indicates that the CZE enantiomers distribute intracellularly in the brain and/or bind readily to tissue components in the extracellular space.

Our results highlight the fact that the measurement of both total and unbound concentrations of drugs in blood and brain is important to differentiate the processes involved in stereoselective brain distribution. The brain distribution of apomorphine enantiomers has been characterized by Sam et al. (1997) in a similar way. There was no difference in the total steady-state concentration of R- and S-apomorphine in either blood or brain, indicating no difference between the two enantiomers regarding their brain distribution. However, measurement of unbound concentrations in brain ISF and blood reveals that the uptake at the BBB was stereoselective. The mefloquine enantiomers were compared in mice with and without the administration of the P-glycoprotein inhibitor elacridar (Barraud de Lagerie et al., 2004). It was concluded that the mefloquine enantiomers undergo efflux at the BBB in a stereoselective manner. Again, the drawback with the measurement of only total concentrations is that the differences in plasma protein binding and the distributional aspect of drugs within the brain cannot be addressed.

The plasma pharmacokinetic parameters of the CZE enantiomers showed that protein binding plays an important role in the blood pharmacokinetics of the enantiomers. The difference in systemic $V$ of the two enantiomers disappeared in $V_a$. The difference in CL was also reduced in CL$_u$, although the CL$_u$ for the two enantiomers was still significantly different (Table 1). The $f_{u}$ for the CZE enantiomers in plasma, determined in vivo by microdialysis in this study (0.50 and 0.15 for S- and R-CZE, respectively), was higher than that determined for the in vitro equilibrium dialysis method in a previous study (manuscript submitted for publication; 0.21 ± 0.04 and 0.10 ± 0.04 for S- and R-CZE, respectively). The reason for this discrepancy was unclear. To confirm the validity of the microdialysis setup used, protein binding in guinea pig plasma was determined in vitro using the same type of microdialysis blood probes (CMA20) according to the method described by Ekbolm et al. (1992). The values of $f_{u}$ obtained for S- and R-CZE were 0.21 ± 0.02 and 0.10 ± 0.01, respectively. Similar results from the two techniques indicated that the higher $f_{u}$ of CZE enantiomers reflects the in vivo situation. The main interaction of CZE in blood is binding to albumin (Bree et al., 2002). It is known that the acute phase reaction in the disease state and injury leads to hypoalbuminemia (Fleck et al., 1985; Sjønness et al., 2001). The albumin levels were compared in naive guinea pigs and guinea pigs that had undergone microdialysis surgery. A standard bromocresol green photometric method was used, and three animals were included in each group. The albumin levels were lower (1.8–2.5%) in plasma of the guinea pigs that had undergone microdialysis surgery compared with naive animals (2.6–3.2%). This reduction in the levels of albumin can explain the higher unbound fraction observed for cetirizine enantiomers in vivo using the microdialysis technique. Thus, protein binding might need to be determined in vivo with microdialysis or ex vivo, alternatively, using plasma from sham-operated animals.

A conclusion regarding stereoselectivity in brain distribution has several components, BBB equilibration, binding in brain tissue and blood, and is dependent on the chosen parameter. Although the differences in $K_{p,u}$ between R- and S-CZE could be interpreted as a stereoselective brain distribution, this is not the case when the determining factors are separated. There is no difference in $K_{p,u}$ i.e., the BBB equilibrium between the enantiomers, showing that there is no enantiomer difference in P-glycoprotein-mediated efflux across the BBB. Nor is there any difference in brain tissue distribution and binding, although both the enantiomers are extensively distributed intracellularly and/or bound to ISF protein in brain. The difference observed in $K_{p}$ is only caused by the difference in plasma protein binding. Thus, when determining stereoselectivity in brain distribution, it is important to study all factors governing this distribution.

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**References**


