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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      CZE, cetirizine; BBB, blood-brain barrier; R-CZE, levocetirizine; S-CZE,

      dextrocetirizine; ISF, interstitial fluid; LC-MS/MS, liquid chromatography

      tandem mass spectrometery; IS, internal standard; AGP, α-acid glycoprotein,

      BSA, bovine serum albumin.

Abstract

The objective of this study was to compare the blood-brain barrier (BBB) transport and brain distribution of levo (R-CZE) and dextrocetirizine (S-CZE). *Methods:* 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 LC-MS/MS. 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 $K_{p,uu}$ compensates for binding also within the brain tissue and directly quantifies the transport across the BBB. $V_{u,br}$ describes binding within the brain. *Results and Conclusions:* 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 as the $K_{p,uu}$ was 0.17 and 0.14 (ns) 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.

Introduction

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 H_1-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 H_1-receptors at given doses (Snowman and Snyder, 1990; Tashiro et al., 2004). P-glycoprotein-mediated efflux at the BBB has been shown to be a reason for the reduced brain penetration of CZE (Chen et al., 2003; Polli et al., 2003).

CZE is a racemic drug and the pharmacological activity of CZE is mainly attributed to levocetirizine (R-CZE) (Devalia et al., 2001). In binding assays, R-CZE has demonstrated a 2-fold higher affinity for the human H_1-receptor compared to 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 H_1-receptors, with R-CZE demonstrating a far longer dissociation half-life (142 min) than S-CZE (6 min) (Gillard et al., 2002). 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. Difference in the penetration of the enantiomers could result in different availability of the enantiomers for the H_1-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 the blood and 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 play a major role in stereoselective brain distribution, if any, of the CZE enantiomers.

The microdialysis technique is being increasingly applied in pharmacokinetic studies to characterize drug transport to the brain *in vivo* (Elmquist 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 along with unbound concentrations will give a measure of binding in blood and brain, respectively.

Knowing that i) R-CZE is the active enantiomer, ii) sedation is correlated with the amount of drug entering into the brain, iii) 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, 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.

Methods

Probes and Chemicals

Racemic CZE, the pure enantiomers S- and R-CZE and an Internal Standard (IS, ucb20028) {2-[2-(4-Benzhydrylidene-piperidin-1-yl)-ethoxy]-ethoxy}-acetic acid chlorhydrate were supplied by UCB Pharma (Braine l'Alleud, Belgium). Isoflurane[®], saline, Ringer (33 mg KCl, 330 mg $CaCl_2.H_2O$, 8.6 g 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 (Sigma Chemical Co., St Louis, MO, U.S.A.). All chemicals were of analytical grade and solvents were of HPLC grade. The water was purified by a Milli-Q Academic system (Millipore, Bedford, MA, USA). Microdialysis probes, CMA/12 (3 mm and 4 mm) and CMA/20 (10 mm), were purchased from CMA/Microdialysis (CMA, Solna, Sweden). The probe membranes were made of polycarbonate and have a molecular weight cutoff of 20 kD.

Animals

The guinea pig was selected as the animal model as they have high brain concentrations of H_1 receptors compared to rats and are often used in antihistamine work (Hill and Young, 1980). Male Dunkin Hartley guinea pigs (n = 8) weighing 350-550 g (Mollegaard Denmark and Charles River, France) were acclimatized for at least 5 days at 22°C and controlled humidity prior to 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 probe membrane. 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 3mm) was first placed in an Eppendorf tube containing blank Ringer

solution for 30 min, followed by Ringer solution containing CZE (50 ng/ml, C_m) 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,

C_{in}) 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 (C_{out}) 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 = C_{out}/C_{in} and the retrodialysis

recovery was determined by the formula, Recovery = $(C_{in}-C_{out})/C_{in}$.

Surgical procedure

The guinea pigs were anaesthetized 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. They were placed on a heating pad to maintain the body temperature at 38°C during surgery. FEP tubings were 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 kD 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 (Davis Kopf Instruments, Tujunga, USA), and a midline incision was made to expose the skull. A hole was drilled in 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 hours later.

Probe Calibration *in vivo*

Non-buffered 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 analyses, 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, Sweden). Samples were collected at 15 min intervals for 60 min.

All the probes were calibrated *in vivo* according to the retrodialysis by drug (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 prior to systemic drug administration. The relative recovery of CZE was estimated in each guinea pig and a mean value of at least 3 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 mg/kg. The maximum total plasma target concentration was 3000 ng/ml. Blood and brain ISF were continuously sampled by microdialysis. During the infusion and 1 hour post-infusion, dialysates were collected every 15 minutes, and then every 30 min for the next 4

hours. Arterial blood samples were collected at 0, 5, 10, 30, 60, 90, 120, 180, 240, 360

minutes and the plasma was separated by centrifugation for 5 minutes 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 an LC-MS/MS method described previously (Gupta et al., 2005). In brief, 50 µl

plasma was precipitated with 100 µl acetonitrile containing IS. A portion of the

supernatant (50 µl) was evaporated, the residue was dissolved in 500 µl the mobile phase

and 50 µl was injected onto the LC-MS/MS system. The chromatographic separation of

the CZE enantiomers was achieved by a Chiral-AGP column (α-acid glycoprotein,

150×4.0 mm, ChromTech, Hägersten, Sweden) tandemed with a triple quadropole mass

spectrometer (Quattro Ultima; Micromass, 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 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 similar way as 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 %)-50 ng/ml for both the enantiomers.

Basic relationships

To characterize brain distribution of the CZE enantiomers, three different partition coefficients K_p, $K_{p,u}$ and $K_{p,uu}$ were calculated. The partition coefficient K_p was calculated as

$$K_p = \frac{AUC_{tot,br,0-\infty}}{AUC_{tot,pl,0-\infty}} \tag{1}$$

where $AUC_{tot,br,0-\infty}$ and $AUC_{tot,pl,0-\infty}$ are the areas under the curve of total concentrations vs. time in brain and plasma, respectively. The K_p can also be expressed as

$$K_p = \frac{f_{u,pl} * AUC_{u,brISF,0-\infty}}{f_{u,brISF} * AUC_{u,pl,0-\infty}} \tag{2}$$

where $AUC_{u,brISF,0-\infty}$ and $AUC_{u,pl,0-\infty}$ are the areas under the curve of unbound concentrations vs. time in brain ISF and plasma, respectively. The $f_{u,pl}$ 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 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 $K_{p,u}$ was calculated as:

$$K_{p,u} = \frac{AUC_{tot,br,0-\infty}}{AUC_{u,pl,0-\infty}} \tag{3}$$

or expressed differently

$$K_{p,u} = \frac{AUC_{u,brISF,0-\infty}}{f_{u,brISF} * AUC_{u,pl,0-\infty}}$$ (4)

Thus $K_{p,u}$ 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 $K_{p,uu}$ and directly describe the

equilibrium across the BBB:

$$K_{p,uu} = \frac{AUC_{u,brISF,0-\infty}}{AUC_{u,pl,0-\infty}}$$ (5)

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 $\delta*f_{u,brISF}$ where δ is the fraction of brain volume in

which the unbound drug is distributed (Fichtl B, 1991). This 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

$$V_{u,br} = \frac{(A_{br} - V_{bl} * C_{bl})}{C_{u,brISF}}$$ (6)

where A_{br} is the total amount of the CZE enantiomers per g brain, V_{bl} is the volume of

blood per g brain, C_{bl} is the total concentration in blood, and $C_{u,brISF}$ is the unbound

concentration is 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 0.12-0.20 ml/g brain (Levin et al., 1970; Goodman et al., 1973). Thus, a

volume close to 0.2 ml*g brain^{-1} shows that the drug is mainly distributed into brain ISF

and a higher value indicates that drug is either distributed intracellularly and/or binds to

protein in ISF.

For cetirizine enantiomers, 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 $V_{u,br}$, (Equation

6) plasma volume and total plasma concentrations were used, with the assumption that

the CZE enantiomers are not or 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 brain (Bosse and Wassermann, 1970). A_{br} was determined

in the whole brain sample taken at 360 min.

Individual plasma clearance (CL) and apparent volume of distribution (V) were

computed as $CL = Dose_{iv}/AUC_{tot,pl, 0-\infty}$ and $V = CL/\lambda$ respectively, where λ 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 microdialysis experiment. The terminal half life in

plasma $(t_{1/2,pl})$ and brain $(t_{1/2,br})$ was expressed as $\ln2/\lambda_{pl}$ and $\ln2/\lambda_{br}$. The λ_{pl} and λ_{br} 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 $AUC_{0-\infty}$ were expressed as the sum

of area under the corresponding concentration versus time curve until the last observation

(AUC_{0-t}) and the residual area $(AUC_{t-\infty})$. The AUC_{0-t} was calculated by the trapezoidal

method. The $AUC_{t-\infty}$ 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, USA) as the sample number was too low to assume normal distribution of

the population parameters. However for the parameters K_p, $K_{p,u}$, $K_{p,uu}$ and $f_{u,pl}$ for S and

R-CZE were compared using paired t-test as 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* recovery of S- and R-CZE

determined by direct dialysis of the brain probes were 48.0 ± 4.59 % and 47.8 ± 4.90 %

and by retrodialysis were 44.9 ± 5.10 % and 44.4 ± 5.11 %, respectively (n.s.). The *in*

vivo recovery 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 Figure 1. The total concentrations of R-CZE in plasma were higher than S-CZE

whereas the unbound concentrations of R-CZE were lower than S-CZE. As in plasma, the

unbound levels of R-CZE in brain ISF were lower than S-CZE. The $AUC_{u,pl,0-\infty}$,

$AUC_{u,brISF,0-\infty}$ and A_{br} were significantly lower for R-CZE than S-CZE (Table 1). The

terminal half-life of 4.9 and 3.9 h for S- and R-CZE, respectively, in brain was

significantly longer than the half-life of 2.2 and 1.9 h for S- and R-CZE in blood (p =

0.008; Table 1).

The parameters describing the brain distribution of the CZE enantiomers are shown in

Figure 2 and 3. The K_p for S- and R-CZE were 0.22 ± 0.05 and 0.04 ± 0.01 indicating

differences in the brain distribution of the two enantiomers. The $K_{p,u}$ was 0.44 ± 0.09 and

0.29 ± 0.07 for S- and R-CZE respectively. Though the difference indicated by the K_p

ratio was reduced, the $K_{p,u}$ values of the two enantiomers were still significantly different

(p = 0.0005). However, the $K_{p,uu}$ value of 0.17 ± 0.06 and 0.14 ±0.04, and $V_{u,br}$ of 2.86 ±

0.88 and 2.39 ± 0.79 ml were not statistically significant between S- and R-CZE,

respectively, indicating no stereoselective brain distribution.

The systemic CL and V derived from total concentration of S- and R-CZE differed

significantly (p = 0.008) with the CL and V of S-CZE being 2.6 and 2.3 times higher than

R-CZE, respectively. There was no significant difference between the V_u of the two

enantiomers whereas the CL_u was significantly higher for R-CZE (p = 0.008). The $f_{u,pl}$ of

S- and R-CZE determined by microdialysis *in vivo* was 0.50 and 0.15, respectively (Table

1).

The residual areas $AUC_{360-\infty}$ were 39-48 % for concentration-time curves in brain and

were 17-21 % for concentration-time curves in blood. We also calculated the three

partition coefficients describing the brain distribution using only AUC_{0-t}. The comparison

of the values for the CZE enantiomers showed similar results (data not shown).

Discussion

This study was designed to compare the CZE enantiomers regarding their brain distribution and exclusively measure and quantify their BBB transport . It was also designed to elucidate the processes that could lead to stereoselectivity in brain distribution. In general, assuming no active process is involved in membrane transport, the distribution of a drug to any tissue depends on its binding in blood and in the tissue. Stereoselectivity in distribution could then be explained by stereoselective binding either to plasma proteins or to tissue components. However the brain is separated from blood by the BBB which consist of various transporter proteins involved in the active influx and efflux of drugs to and from the brain. Thus the transport process across the BBB could also be a stereoselective process, leading to differences in brain distribution.

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. As K_p compares total brain concentration to total plasma concentration this measure includes all the three factors governing the distribution; protein binding in blood, binding to brain parenchymal cells and proteins, and transport across the BBB (Equation 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 50-fold range in absolute K_p values were 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 $K_{p,u}$ accounts for the differences in binding to blood components (Equation 4). The plasma protein binding of CZE enantiomers was found to be stereoselective, with an f_u of 0.50 and 0.15 for S- and R-CZE, respectively. The $K_{p,u}$ of 0.44 for S-CZE against 0.29 of R-CZE indicates that the stereoselectivity in brain partitioning observed with K_p is mainly explained by the difference in plasma protein 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 till 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.

Since $K_{p,uu}$ is calculated from the unbound concentrations on both sides of the BBB (Equation 5) it more closely describes the transport across the BBB independent of binding in the blood and brain (Hammarlund-Udenaes et al., 1997), and can therefore directly measure any stereoselectivity in active transport across the BBB. The $K_{p,uu}$ was 0.17 and 0.14 for S- and R-CZE, respectively. A $K_{p,uu} < 1$ 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. and Polli et al. (Chen et al., 2003; Polli et al., 2003). The similar $K_{p,uu}$ for S- and R-CZE indicates 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 fits with the results from Whomsley et al., 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 $V_{u,br}$. 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

$V_{u,br}$ of 2.86 ml/g brain for S-CZE was not significantly different from 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

Equation 4. The $K_{p,u}$ is a product of $V_{u,br}$ $(1/f_{u,brISF})$ and $K_{p,uu}$. The $V_{u,br}$ and $K_{p,uu}$ are not

different for the two enantiomers, however there is trend of 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 $V_{u,br}$ 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 and co-workers in a similar way (Sam et al.,

1997). There was no difference in the total steady state concentration of R- and S-

apomorphine in both blood and 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_u. 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 with *in vitro* equilibrium dialysis method in a previous study (paper

submitted; 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.

(Ekblom 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 acute phase reaction in

disease state and injury lead to hypoalbuminaemia (Fleck et al., 1985; Sjunnesson et al.,

2001). The albumin levels were compared in naïve 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 as

compared to naïve 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

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. While the differences in K_p and $K_{p,u}$ 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 $K_{p,uu}$, i.e. the BBB

equilibrium between the enantiomers, showing that there is no enantiomeric differences

in P-glycoprotein mediated efflux across the BBB. There is neither 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 difference in plasma protein binding. Thus, when determining

stereoselectivity in brain distribution it is important to study all factors governing this

distribution.

Acknowledgments

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Footnotes

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Legends to Figures

Figures 1

Total-concentration time profile in blood and unbound-concentration time profiles

(average ± SD) in blood and brain ISF of levo (R-CZE) and dextrocetirizine (S-CZE)

obtained from regular blood sampling and microdialysis, respectively. The data was

collected during a 1-hour constant rate infusion of racemic cetirizine to eight guinea pigs

and five hours after the end of infusion. All the unbound concentrations are corrected for

recovery obtained by the retrodialysis by drug method before drug administration.

Figures 2

The three partition coefficient K_p, $K_{p,u}$, $K_{p,uu}$ of S- and R-CZE (average ± SD, n = 8)

measuring brain distribution of the enantiomers in guinea pigs. Both the K_p and $K_{p,u}$ for

S-CZE was significantly higher than R-CZE (p < 0.01).

Figures 3

Unbound volume of distribution, $V_{u,br}$ of S- and R-CZE in brain (average ± SD, n = 8).

The $V_{u,br}$ is measure of binding within the brain.

Table 1: Pharmacokinetic parameters in blood and brain of S- and R-CZE in guinea pigs

(n = 8) after intravenous constant rate infusion of racemic CZE. The results are presented

as average ± SD. Statistical analysis was performed using Wilcoxon's signed rank test.

Parameter	S-CZE	R-CZE	Ratio S/R
Cl (ml/min/kg)	7.29 ± 1.2	2.78 ± 0.62	2.6**
Cl_u (ml/min/kg)	14.9 ± 2.10	18.8 ± 3.67	0.8**
V (ml/kg)	1385 ± 277	593 ± 151	2.3**
V_u (ml/kg)	2808 ± 602	3028 ± 578	0.9
$f_{u\,pl}$	0.50 ± 0.10	0.15 ± 0.03	3.3***
$t_{1/2,pl}$ (h)	2.20 ± 0.45[a]	1.90 ± 0.45[b]	1.2**
$t_{1/2,br}$ (h)	4.94 ± 1.40[a]	3.94 ± 1.63[b]	1.3*
$AUC_{u,pl,0-\infty}$ (min*ng/mL)[c]	68510 ± 9104	55545 ± 9576	1.2**
$AUC_{u,brISF,0-\infty}$ (min*ng/mL)[c]	11435 ± 2843	7498 ± 1728	1.5**
A_{br} (ng/g brain)[d]	45.9 ± 13.3	25.4 ± 9.0	1.8**

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

and R-CZE

[a] (p <0.01; statistical comparison of half life in blood and brain for S-CZE)

[b] (p <0.01; statistical comparison of half life in blood and brain for R-CZE)

[c] Dose normalized AUC

[d] Total amount per g brain at 360 min after start of the infusion corrected for plasma
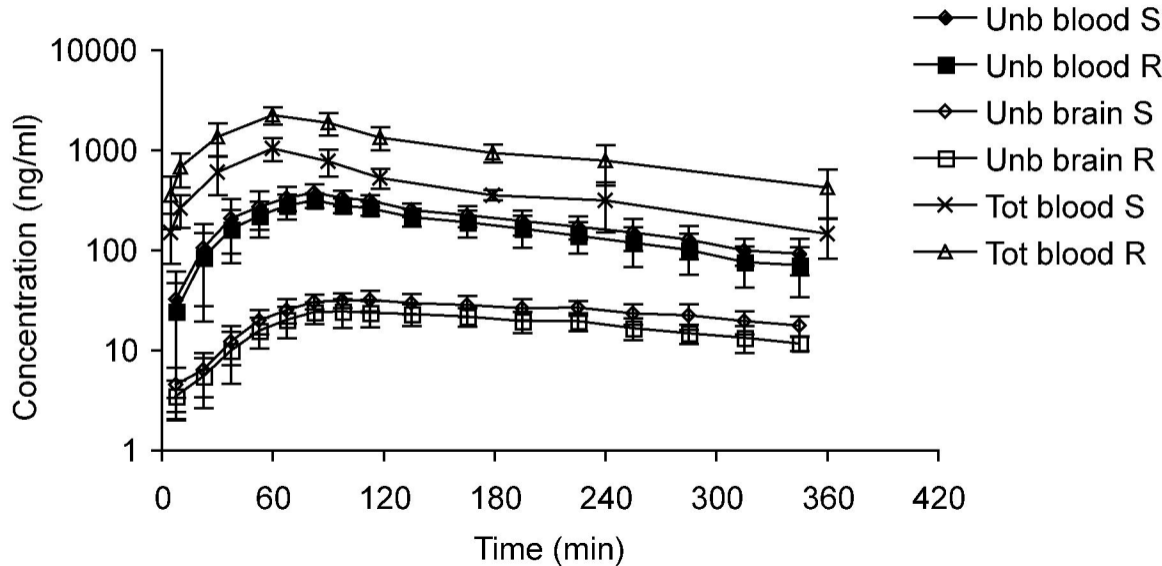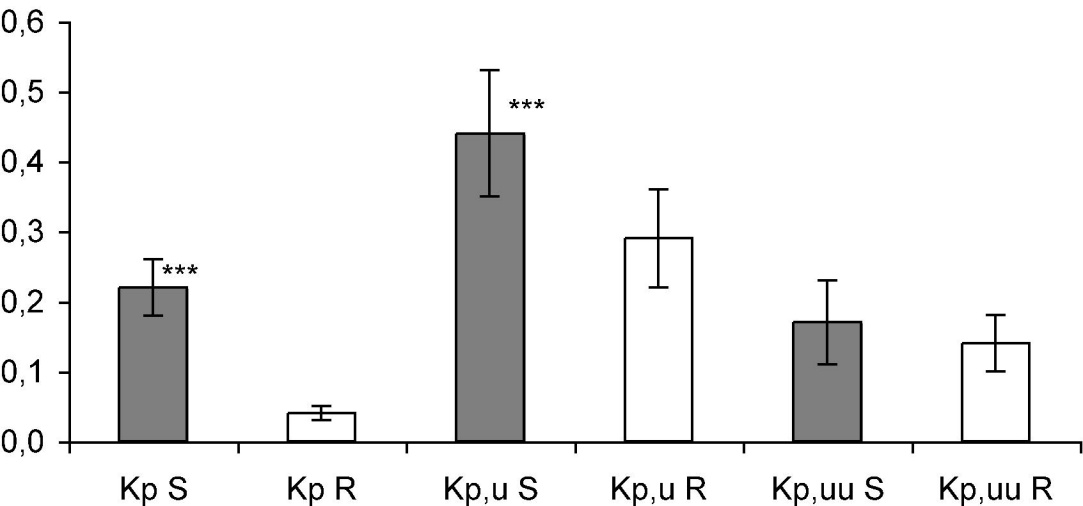volume in the brain

Figure 1

Figure 2

Figure 3