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ORIGINAL ARTICLE

Disposition of eslicarbazepine acetate in the mouse after oral administration

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ABSTRACT

Eslicarbazepine acetate is a promising antiepileptic drug structurally related to carbamazepine and oxcarbazepine, which is in the final phase of clinical development. The metabolism of eslicarbazepine acetate is clearly species dependent and, in this case, among small laboratory animals, the mouse seems to be the most relevant species to humans. Hence, the aim of this study was to investigate the plasma, brain and liver disposition of eslicarbazepine acetate in mice to better understand its disposition in humans. Adult male CD-1 mice were treated orally with a single dose of eslicarbazepine acetate 350 mg/kg. Blood samples, brain and liver tissues were taken at 0.25, 0.5, 0.75, 1, 2, 4, 6, 10, 16 and 24 h post-dose. Plasma and tissue levels of eslicarbazepine acetate and its metabolites (S-licarbazepine, R-licarbazepine and oxcarbazepine) were assessed by using high-performance liquid chromatography-ultraviolet detection. Both eslicarbazepine acetate and R-licarbazepine concentrations were below the limit of quantification of the assay in all matrices. Eslicarbazepine acetate was rapidly and extensively metabolized to S-licarbazepine (major metabolite), which was oxidized to oxcarbazepine to a small extent. The brain/plasma ratios suggest that the brain exposure to S-licarbazepine and oxcarbazepine was approximately 30% of their total systemic exposure. However, S-licarbazepine crossed the blood–brain barrier (BBB) less efficiently than oxcarbazepine. On the other hand, the liver/plasma ratios support the notion that S-licarbazepine undergoes hepatic accumulation, whereas oxcarbazepine appears to leave this compartment twice as fast as S-licarbazepine. Thus, the diffusion through the BBB is favourable to oxcarbazepine and the liver acts like a deposit of the pharmacologically active metabolite of eslicarbazepine acetate (S-licarbazepine).

INTRODUCTION

Epilepsy is one of the most common neurological disorders affecting about 1% of the population worldwide [1,2]. Studies in paediatric and adult epileptic patients have shown that the lack of seizure control and seizure severity has a negative impact on their quality of life [3,4]. Unfortunately, despite the increasing availability of new antiepileptic drugs, almost one-third of patients continue to present seizures that appear to be resistant to all pharmacological schemes [5,6]. Therefore, to develop new and more effective antiepileptic drugs, a lot of compounds are currently undergoing preclinical and clinical tests [7].

Eslicarbazepine acetate or S-(−)-10-acetoxy-10,11-dihydro-5H-dibenz/b,f/azepine-5-carboxamide, previously known as BIA 2-093, is a novel central nervous system (CNS)-active compound completing phase III

clinical trials (as add-on therapy in refractory partial epilepsy) and undergoing phase II clinical trials (as monotherapy in partial epilepsy and in bipolar disorder) [8]. Chemically, it shares with carbamazepine and oxcarbazepine the dibenzazepine nucleus bearing the 5-carboxamide substituent, but is structurally different at the 10,11-position (*Figure 1*) [9]. These molecular differences possibly are on the basis of their different metabolic profiles. In humans, whereas carbamazepine undergoes oxidative metabolism to carbamazepine-10,11-epoxide, oxcarbazepine is rapidly reduced to its pharmacologically active licarbazepine metabolite (10,11-dihydro-10-hydroxy-carbamazepine) avoiding the formation of epoxides [10–12]. Oxcarbazepine is an achiral prodrug, but its biotransformation to licarbazepine was shown to be stereoselective appearing in plasma as S-licarbazepine and R-licarbazepine in approximately 4 : 1 enantiomeric ratio [13–15]. On the other hand, eslicarbazepine acetate is quickly and extensively metabolized to S-licarbazepine (95–98%) and, to a very small extent, to R-licarbazepine and oxcarbazepine [11,16,17]. Specifically, taking S-licarbazepine and R-licarbazepine into account, some in vivo studies in rodents have indicated that both enantiomers have a comparable anticonvulsant activity, R-licarbazepine being only slightly less active [18,19]. Nevertheless, from the work published by Benes et al. [9] it is clear that eslicarbazepine acetate is a more potent anticonvulsant than oxcarbazepine. At first sight, this finding would be unexpected, because they are the parent drugs of S- and R-licarbazepine. Likewise, after intravenous infusion of the racemic mixture of S- and R-licarbazepine to healthy adult volunteers, the area under the concentration–time curve (AUC) of S-licarbazepine was found to be 40% higher than that of R-licarbazepine [20], suggesting pharmacokinetic differences in the disposition of both enantiomers. Actually, it is recognized that each enantiomer may have different pharmacological effects in biological systems [21].

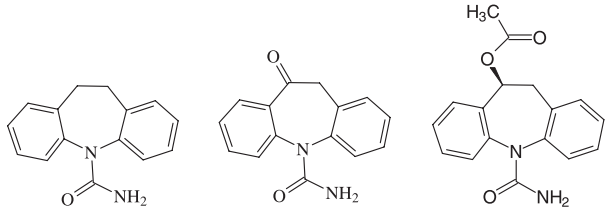

Figure 1 Chemical structures of carbamazepine (CBZ), oxcarbazepine (OXC) and eslicarbazepine acetate (ESL).

Thus, eslicarbazepine acetate was specifically designed to circumvent its further biotransformation to toxic metabolites, such as epoxides, and to avoid the enantiomeric impurity and unnecessary production of enantiomers or diastereoisomers of its metabolites, without losing anticonvulsant potency [9,22]. Metabolic studies in rats, mice and rabbits showed that eslicarbazepine acetate is rapidly hydrolysed to S-licarbazepine, which in rats undergoes a fast oxidation to oxcarbazepine [22]. As a result, bearing in mind the preliminary studies performed by Hainzl et al. [22] and the pharmacokinetic data derived from clinical trials of eslicarbazepine acetate [8,11,16,17], it appears to be overt that in this case, among small laboratory animals, the mouse seems to be the best whole animal model to better understand the eslicarbazepine acetate disposition in humans. Indeed, using carboxymethylcellulose as a carrier vehicle, the plasma disposition of eslicarbazepine acetate in CD-1 mice after oral gavage was comparable with that occurring in humans [8,22]. Therefore, the aim of this study was to describe the plasma, brain and liver disposition of eslicarbazepine acetate and its metabolites in CD-1 mice following a single oral administration.

MATERIALS AND METHODS

Drugs

Eslicarbazepine acetate (100% pure by using high-performance liquid chromatography, HPLC), S-licarbazepine (99.79% pure by using HPLC), R-licarbazepine (100% pure by using HPLC), oxcarbazepine (>98% pure by using HPLC) and BIA 2–265 [10-(hydroxymethyl)-10-nitro-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide (97.4% pure by using HPLC, used as internal standard)] were supplied by BIAL (Porto, Portugal). Carboxymethylcellulose was purchased from Sigma (St Louis, MO, USA).

Animals and experimental design

Adult male CD-1 mice obtained from Harlan-Interfauna (Barcelona, Spain) weighing 30–35 g were used. The animals were housed in local animal facilities with light (12 h : 12 h light/dark cycle)- and temperature (22 ± 1 °C)-controlled environment for at least 5 days prior to the experiments. A regular chow diet (4RF21; Mucedola, Milan, Italy) and tap water were available ad libitum, before and immediately after the drug administration.

Eslicarbazepine acetate was suspended in a 0.5% carboxymethylcellulose aqueous solution and all mice were treated by using oral gavage (0.5 mL/30 g mouse

weight) with a single dose of 350 mg/kg. Blood samples, brain and liver tissues were taken at 0.25, 0.5, 0.75, 1, 2, 4, 6, 10, 16 and 24 h post-dose (eight mice were used at each time point). Blood samples were collected into heparinized tubes by decapitation preceded by cervical dislocation. The plasma was separated by centrifugation at 1800 ***g*** for 10 min (4 °C) and stored at −30 °C until analysis. After exsanguination, the brain and liver were quickly removed, weighed and then homogenized (1 ***g***/4 mL) in a 0.1 M sodium phosphate buffer (pH 5). The tissue homogenates were centrifuged at 2600 ***g*** for 15 min (4 °C) and the supernatants were also stored at −30 °C until analysis.

All animal experiments were conducted in accordance with the European Directive (86/609/EEC) for the accommodation and care of laboratory animals and the experimental procedures were approved by the Portuguese Veterinary General Division.

Assay of eslicarbazepine acetate and metabolites

Plasma and tissue concentrations of eslicarbazepine acetate and its metabolites were determined by using a validated enantioselective high-performance liquid chromatography-ultraviolet detection-solid phase extraction (HPLC-UV-SPE) assay [23]. Briefly, an aliquot of each plasma sample (250 μL) was added to 750 μL of 0.1 M sodium phosphate buffer (pH 5) spiked with 2 μg of the internal standard. The samples were vortex mixed and loaded into Waters (Milford, MA, USA) Oasis® HLB extraction cartridges (30 mg, 1 mL), which were previously conditioned with 1 mL of methanol, 1 mL of acetonitrile and 1 mL of water/acetonitrile (95 : 5 v/v). After sample elution, the loaded cartridges were submitted to −30 kPa and washed twice with 1 mL of water and twice with 1 mL of water/acetonitrile (95 : 5 v/v). After drying the sorbent under airflow for 5 min, the drugs were eluted with 1 mL of ethyl acetate under gentle vacuum and then the cartridges were dried for 30 s at −30 kPa. The eluates were evaporated to dryness under a nitrogen stream at 45 °C and the residues reconstituted in 100 μL of water/methanol (88 : 12 v/v), vortexed for approximately 30 s and placed in an ultrasonic bath at room temperature for approximately 1 min. Following this, the reconstituted extracts were transferred to 0.22-μm Spin-X (Costar®; Corning Inc., Corning, NY, USA) centrifugal filters, centrifuged at 9000 ***g*** for 2 min and 20 μL of the final filtered extract was used for HPLC analysis.

The supernatants of the brain and liver homogenates were centrifuged (9000 ***g*** for 20 min) for a second time to give clear supernatants of which 1 mL (∼250 μg of tissue) was also spiked with 2 μg of the internal standard. Afterwards, the drugs in the brain and liver supernatants were extracted by using the solid phase extraction procedure already described for plasma samples, with some differences in the washing steps and vacuum conditions (−40 kPa). The loaded cartridges were washed with 1 mL of water and 1 mL of water/acetonitrile (95 : 5 v/v) for three or four times for the brain and liver samples respectively.

The HPLC analysis was performed on a BAS-480 Liquid Chromatograph equipped with a PM-80 pump, a Rheodyne manual injector with a 20-μL loop, a BAS UV-116 UV–Vis detector, a BAS LC-22C Temperature Controller, a BAS DA-5 Chromatography Control and a Data System Interface (all from Bioanalytical Systems, West Lafayette, IN, USA). Data collection and integration were achieved by means of a BAS Chromgraph Control and Chromgraph Report software version 2.30 (Bioanalytical Systems, West Lafayette, IN, USA). The chromatographic separation was carried out at 30 °C by isocratic elution with water/methanol (88 : 12 v/v), at a flow rate of 0.7 mL/min, on a LiChroCART 250–4 ChiraDex (β-cyclodextrin, 5 μm) column protected by using a LiChroCART 4–4 ChiraDex (β-cyclodextrin, 5 μm) guard column purchased from Merck KGaA (Darmstadt, Germany). The detector was set at 225 nm and the run time was 28 min. The method was linear ($r > 0.994$) for eslicarbazepine acetate and oxcarbazepine over concentration ranges 0.4–8 μg/mL in the mouse plasma, 0.1–1.5 μg/mL in the supernatant of the brain homogenate and 0.1–2 μg/mL in the supernatant of the liver homogenate, and for each licarbazepine enantiomer in the ranges of 0.4–80, 0.1–15 and 0.1–20 μg/mL in the plasma, brain and liver respectively. The precision and accuracy were lower than 15%. The mean recovery of the extraction method ranged from 89% to 102% taking eslicarbazepine acetate and its metabolites into account. No peaks resulting from the plasma and tissues interfered at the retention time of the analytes. The limit of quantification (LOQ) was 0.4 μg/mL in the plasma and 0.1 μg/mL (∼0.4 μg/g) in the supernatant of tissue homogenates.

Pharmacokinetic analysis

The peak concentration (C_{max}) of eslicarbazepine acetate metabolites in the plasma and tissues and the time to reach C_{max} (t_{max}) were derived directly from the measured concentration values. Other pharmacokinetic parameters were estimated from mean concentration

data (n = 8) obtained at each time point by using non-compartmental analysis using the WinNonlin® version 4.1 (Pharsight Co, Mountain View, CA, USA): AUC from time zero to the last sampling time at which concentrations were at or above the limit of quantification (AUC_{0-t}) is calculated by using the linear trapezoidal rule; AUC from time zero to infinity ($AUC_{0-\infty}$) is calculated from $AUC_{0-t} + (C_{last}/\lambda_z)$, where C_{last} is the last quantifiable concentration and λ_z is the apparent terminal rate constant calculated by using log-linear regression of the terminal segment of the concentration–time profile; apparent terminal elimination half-life ($t_{1/2}$) and mean residence time (MRT). Mean plasma and tissue concentrations lower than the LOQ of the assay were taken as zero for all calculations.

RESULTS

Plasma eslicarbazepine acetate disposition

The mean plasma concentration–time profiles of eslicarbazepine acetate and its metabolites (S-licarbazepine, R-licarbazepine and oxcarbazepine) after a single oral dose of 350 mg/kg eslicarbazepine acetate in the mouse are depicted in *Figure 2*. Plasma pharmacokinetic parameters of the parent compound eslicarbazepine acetate and R-licarbazepine could not be calculated, because eslicarbazepine acetate was not detected in any samples and R-licarbazepine, when detected, appeared below the LOQ of the assay. The metabolites of eslicarbazepine acetate that were present in the plasma in measurable amounts were S-licarbazepine and oxcarbazepine, and their corresponding pharmacokinetic parameters estimated by using non-compartmental analysis are given in *Table I*. S-licarbazepine was shown to be the major metabolite in plasma, which is evidenced by the $C_{max}plasma_{S\text{-licarbazepine}}/C_{max}plasma_{oxcarbazepine}$ and $AUC_{0-\infty}plasma_{S\text{-licarbazepine}}/AUC_{0-\infty}plasma_{oxcarbazepine}$ ratios far greater than the unit, 8.47 and 5.67 respectively. Thereby, as assessed by using $AUC_{0-\infty}$, S-licarbazepine represented 85% of total systemic drug exposure, whereas oxcarbazepine corresponded to approximately 15%. The C_{max} for S-licarbazepine and oxcarbazepine were found, respectively, at 0.25 and 1 h after dosing. Plasma concentrations of S-licarbazepine and oxcarbazepine declined with a $t_{1/2}$ of 6.54 and 9.24 h, respectively, and, consequently, comparing the MRT of both compounds, it is apparent that oxcarbazepine remains in the plasma in its unchanged form for a longer period of time than S-licarbazepine (*Table I*).

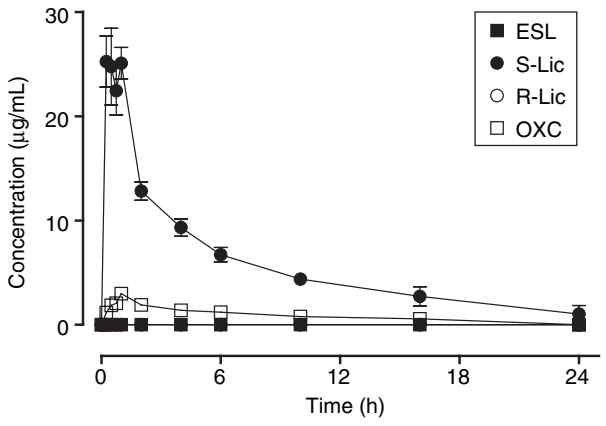

Figure 2 Plasma concentration–time profiles of eslicarbazepine acetate (ESL), S-licarbazepine (S-Lic), R-licarbazepine (R-Lic) and oxcarbazepine (OXC) following a single oral administration of ESL (350 mg/kg). Symbols represent the mean values ± SEM of eight determinations per time point (n = 8 mice).

Table I Plasma, brain and liver pharmacokinetic parameters of eslicarbazepine acetate (ESL) and its metabolites, S-licarbazepine (S-Lic), R-licarbazepine (R-Lic) and oxcarbazepine (OXC), in mice after a single oral dose of ESL 350 mg/kg.

Pharmacokinetic parameters	ESL	S-Lic	R-Lic	OXC
Plasma				
t_{max} (h)	NA	0.25	NA	1.00
C_{max} (µg/mL)	NA	25.25	NA	2.98
AUC_{0-t} (µg h/mL)	ND	136.87	ND	18.14
$AUC_{0-\infty}$ (µg h/mL)	ND	146.51	ND	25.84
$t_{1/2}$ (h)	ND	6.54	ND	9.24
MRT (h)	ND	8.21	ND	12.90
Brain				
t_{max} (h)	NA	1.00	NA	1.00
C_{max} (µg/g)	NA	13.13	NA	3.12
AUC_{0-t} (µg h/g)	ND	40.13	ND	6.76
$AUC_{0-\infty}$ (µg h/g)	ND	45.61	ND	8.07
$t_{1/2}$ (h)	ND	6.30	ND	2.04
MRT (h)	ND	6.96	ND	3.43
Liver				
t_{max} (h)	NA	1.00	NA	0.50
C_{max} (µg/g)	NA	44.15	NA	2.40
AUC_{0-t} (µg h/g)	ND	198.22	ND	11.65
$AUC_{0-\infty}$ (µg h/g)	ND	205.71	ND	15.66
$t_{1/2}$ (h)	ND	5.30	ND	4.46
MRT (h)	ND	6.70	ND	6.70

C_{max} and t_{max} are experimental values: AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$ and MRT values were calculated by using non-compartmental analysis from mean concentrations at each time point (n = 8 mice per group). NA, not available; ND, not determined.

Brain eslicarbazepine acetate disposition and blood–brain distribution

Eslicarbazepine acetate or its active metabolites, in common with other CNS drugs, need to cross the blood–brain barrier (BBB) to exert their therapeutic effect. Therefore, the brain levels of eslicarbazepine acetate and its metabolites were also determined in mice after oral dosing with eslicarbazepine acetate 350 mg/kg (*Figure 3*). The corresponding brain pharmacokinetic parameters calculated by using non-compartmental analysis are summarized in *Table I*. In agreement with the plasma data, only S-licarbazepine and oxcarbazepine were found in quantifiable amounts in the brain, representing S-licarbazepine 85% and oxcarbazepine 15% of total brain drug exposure (assessed by using $AUC_{0-\infty}$). Indeed, the $AUC_{0-\infty}brain_{S\text{-licarbazepine}}/AUC_{0-\infty}brain_{oxcarbazepine}$ ratio was 5.65, similar to that in the plasma.

To assess the extent of S-licarbazepine and oxcarbazepine distribution to the target tissue, their $AUC_{0-\infty}$ brain/plasma ratios were calculated, being 0.31 for both S-licarbazepine and oxcarbazepine. These results support the observation that the distribution of S-licarbazepine and oxcarbazepine into the brain occurs in the same proportion. However, when other parameters such as C_{max}, t_{max}, $t_{1/2}$ and MRT are considered, it is apparent that S-licarbazepine crosses the BBB more slowly than oxcarbazepine in both directions. First, the relative delay of S-licarbazepine in accessing the brain was evidenced by using the $C_{max}brain_{S\text{-licarbazepine}}/C_{max}plasma_{S\text{-licarbazepine}}$ ratio (0.52), which was found to be half of that obtained for oxcarbazepine (1.05). In addition, the S-licarbazepine t_{max} in the brain was displaced to the right comparatively with that in the plasma (from 0.25 to 1 h), whereas the oxcarbazepine t_{max} were coincident in both matrices (1 h). On the other hand, taking into account the values of $t_{1/2}$ and MRT in the brain, it is clear that S-licarbazepine leaves the brain less promptly than oxcarbazepine. Indeed, assuming that the metabolism of oxcarbazepine is irrelevant in the brain tissue, its short $t_{1/2}$ (2.04 h) and MRT (3.43 h) estimated suggest that oxcarbazepine undergoes a high efflux rate from the brain.

Liver eslicarbazepine acetate disposition

The mean liver concentration–time plots of eslicarbazepine acetate and its metabolites are shown in *Figure 4*. Again, the liver pharmacokinetic parameters were estimated by using non-compartmental analysis only for S-licarbazepine and oxcarbazepine, because eslicarbazepine acetate was not detected and R-licarbazepine concentrations were lower than the LOQ (*Table I*). The liver pharmacokinetic profiles obtained in mice, following oral treatment with eslicarbazepine acetate, demonstrated that S-licarbazepine was undoubtedly the main metabolite, corresponding to approximately 93% of overall hepatic drug exposure, while oxcarbazepine was responsible for approximately 7% (assessed by $AUC_{0-\infty}$). The $C_{max}liver_{S\text{-licarbazepine}}/C_{max}liver_{oxcarbazepine}$ and $AUC_{0-\infty}liver_{S\text{-licarbazepine}}/AUC_{0-\infty}liver_{oxcarbazepine}$ ratios were 18.4

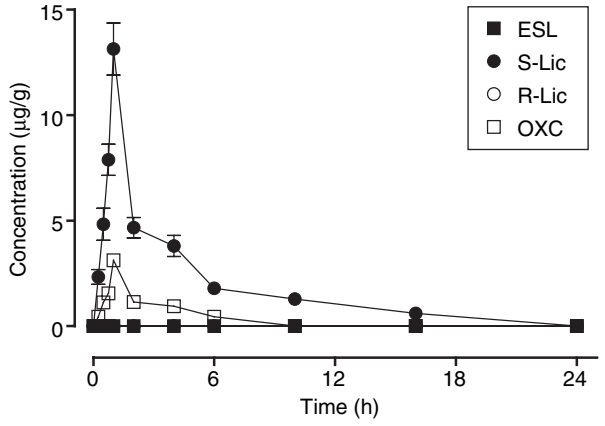

Figure 3 Brain concentration–time profiles of eslicarbazepine acetate (ESL), S-licarbazepine (S-Lic), R-licarbazepine (R-Lic) and oxcarbazepine (OXC) following a single oral administration of ESL (350 mg/kg). Symbols represent the mean values ± SEM of eight determinations per time point (n = 8 mice).

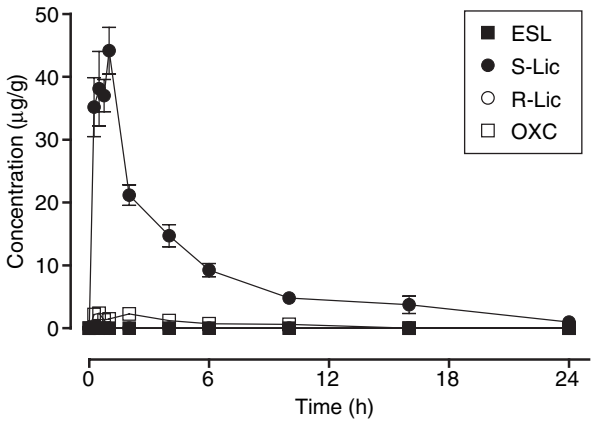

Figure 4 Liver concentration–time profiles of eslicarbazepine acetate (ESL), S-licarbazepine (S-Lic), R-licarbazepine (R-Lic) and oxcarbazepine (OXC) following a single oral administration of ESL (350 mg/kg). Symbols represent the mean values ± SEM of eight determinations per time point (n = 8 mice).

and 13.1 respectively. Comparing the liver and plasma pharmacokinetic profiles, the C_{max} and $AUC_{0-\infty}$ of S-licarbazepine in the liver were 1.75- and 1.40-fold greater than that in the plasma, but at the same time the C_{max} and $AUC_{0-\infty}$ of oxcarbazepine were lower in the liver with $C_{max}liver_{oxcarbazepine}/C_{max}plasma_{oxcarbazepine}$ and $AUC_{0-\infty}liver_{oxcarbazepine}/AUC_{0-\infty}plasma_{oxcarbazepine}$ ratios of 0.80 and 0.61 respectively. The liver S-licarbazepine t_{max} (1 h) was attained later than in the plasma (0.25 h), but at 0.25 h post-dose the liver S-licarbazepine levels were already 80% of the C_{max}. The liver oxcarbazepine t_{max} (0.50 h) occurred earlier than that in the plasma (1 h). MRT and $t_{1/2}$ values estimated in the liver for oxcarbazepine and S-licarbazepine were similar with values of 6.70 and 4.46 h for the first compound and with values of 6.70 and 5.30 h for the second compound respectively. However, striking differences between plasma and liver $t_{1/2}$ and MRT values were observed, particularly for oxcarbazepine, which were approximately two times greater in the plasma.

DISCUSSION

Eslicarbazepine acetate is a promising CNS drug in the final phase of clinical development [8,24], which was selected from a series of new compounds structurally related to carbamazepine and oxcarbazepine [9]. This choice was formerly supported by its potent anticonvulsant activity and low neurotoxicity in rats [9]. Thereafter, as commonly happens with new chemical entities, the eslicarbazepine acetate preclinical development involved studies in several animal species to confirm its efficacy and safety and derive metabolic and pharmacokinetic data to predict the appropriate dosage regimes to entry into humans [22,25]. For ethical and practical reasons, tissue distribution experiments cannot be performed in humans and whole animal models may provide the required data.

This study was designed to characterize the plasma, brain and liver disposition of eslicarbazepine acetate in adult male CD-1 mice, avoiding in this manner the menstrual cycle hormonal interferences and assuring the maturation of all biological systems. First, the brain is the therapeutic target of eslicarbazepine acetate or of its active metabolites and the knowledge of their brain uptake extent will be a good approach to understand this process in humans. On the other hand, it is essential to study the relationship between the liver and plasma levels to recognize the influence of hepatic tissue distribution upon plasma pharmacokinetic profiles.

In the present study, mice were not subjected to any fasting period, which is usual in this type of studies involving oral administration of drugs. Actually, in humans, the presence of food did not change the plasma pharmacokinetic profiles of eslicarbazepine acetate metabolites [26] and by this way the well-being of the animals was preserved. Mice were administered orally with a single dose of eslicarbazepine acetate 350 mg/kg; previous studies revealed that this dose is associated with no significant toxicity and provided a systemic exposure to the main active metabolite (S-licarbazepine) comparable with that occurring in human healthy volunteers and epileptic patients after treatment with therapeutic doses [8,24]. Moreover, the S-licarbazepine plasma levels herein obtained in mice are within the therapeutic range clinically defined for licarbazepine (15–35 μg/mL) following oxcarbazepine administration [27].

The results here reported definitely show that eslicarbazepine acetate in mice is rapidly and extensively metabolized after oral administration, which was evidenced by the lack of detection of the parent drug (eslicarbazepine acetate) at the first sampling point (0.25 h). By contrast, at this time point, large amounts of S-licarbazepine were found in the plasma and liver. Therefore, eslicarbazepine acetate appears to be rapidly absorbed from the gastrointestinal tract and its deacetylation occurs more or less instantly, somewhere in between the gastrointestinal tract and the liver. Some in vitro studies showed that the hydrolysis of eslicarbazepine acetate is non-enzymatic and may be detected almost instantaneously in the rat plasma [22]. At this point, as the enterocytes can play an important role in the metabolism of drugs when passing through the intestinal mucosa, further studies employing everted gut sacs may clarify the intestinal contribute to the extensive first-pass metabolism of eslicarbazepine acetate [28–30].

Overall, taking into consideration the mouse plasma data and that reported from clinical trials following oral administration of eslicarbazepine acetate [17,26], it is evident that the metabolism of eslicarbazepine acetate in humans produces basically the same metabolites as that in the mouse, even though differences exist in their ratios. In humans, the S-licarbazepine/oxcarbazepine plasma ratios were higher than 100 [11], whereas in mice S-licarbazepine/oxcarbazepine plasma ratios were nearly 6. In the rat, the S-licarbazepine/oxcarbazepine plasma ratios were shown to be approximately 0.12, illustrating the predominance of oxcarbazepine in the rat

plasma [22]. Hence, the metabolism of eslicarbazepine acetate is clearly species dependent, being the mouse in this particular case, among small laboratory animals, the most relevant species to humans.

The plasma and liver pharmacokinetic profiles are comparable after oral administration of eslicarbazepine acetate to mice, even though some specific points deserve to be discussed. Our results suggest that S-licarbazepine undergoes hepatic accumulation, while the concentrations of oxcarbazepine increase in the plasma. The higher levels of oxcarbazepine in the plasma may result from its enhanced extrusion from the hepatocytes or by production at sites other than the liver. Assuming that the liver acts as a reservoir for S-licarbazepine, it may be clinically useful to maintain S-licarbazepine plasma concentrations and, consequently, to lengthen the brain exposure to the pharmacologically active metabolite (S-licarbazepine). Neither in the liver nor in the plasma was possible to quantify R-licarbazepine concentrations (below the LOQ), but it was detected at first time points.

The analysis of the mouse plasma and brain pharmacokinetic data reveals that the extent of brain drug exposure to S-licarbazepine and oxcarbazepine corresponded to approximately 30% of the total systemic exposure. Thus, it is obvious that the BBB restricts the penetration of S-licarbazepine and oxcarbazepine into the brain. However, S-licarbazepine has apparently more considerable difficulties than oxcarbazepine in entering and leaving the brain. From this work, it is not easy to explain the differences between S-licarbazepine and oxcarbazepine in crossing the BBB, but their different lipophilicity may be involved. Nevertheless, the brain levels of S-licarbazepine and oxcarbazepine should be influenced by mechanisms other than simple passive diffusion, as Clinckers et al. [31] indicated that oxcarbazepine is a substrate for multidrug transporters and Marchi et al. [32] suggested that licarbazepine is a substrate of P-glycoprotein at the BBB. Accordingly, additional information may be obtained from studies using brain microdialysis, which will allow one to assess the brain penetration of the unbound or pharmacologically active moieties of these compounds [33,34].

Therefore, considering the data presented here, it appears that eslicarbazepine acetate undergoes rapid and extensive conversion to S-licarbazepine, which is then oxidized to oxcarbazepine to a small extent. However, the origin of R-licarbazepine detected in mice remains unknown. In humans, oxcarbazepine clearly undergoes a reductive metabolism to S-licarbazepine and R-licarbazepine, representing the R-enantiomer approximately 20% of the total exposure [13–15]. Accordingly, oxcarbazepine could be pointed out as the possible source of R-licarbazepine detected in mice. However, the low concentrations of oxcarbazepine and the inability of mice to perform its reduction effectively [22] suggest that R-licarbazepine may be produced through a different metabolic pathway. Thus, further studies are needed to define the origin of R-licarbazepine in mice. In this context, it may be useful to investigate the eventual racemization of licarbazepine following the administration of pure enantiomers.

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