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Title

Intranasal administration of carbamazepine to mice: a direct delivery pathway for brain targeting

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Abstract

The currently available antiepileptic drugs are typically administered via oral or intravenous (IV) routes which commonly exhibit high systemic distribution into non-targeted tissues, leading to peripheral adverse effects and limited brain uptake. In order to improve the efficacy and tolerability of the antiepileptic drug therapy, alternative administration strategies have been investigated. The purpose of the present study was to assess the pharmacokinetics of carbamazepine administered via intranasal (IN) and IV routes to mice, and to investigate whether a direct transport of the drug from nose to brain could be involved. The similar pharmacokinetic profiles obtained in all matrices following both administration routes indicate that, after IN delivery, carbamazepine reaches quickly and extensively the bloodstream, achieving the brain predominantly via systemic circulation. However, the uneven biodistribution of carbamazepine through the brain regions with higher concentrations in the olfactory bulb and frontal cortex following IN instillation, in comparison with the homogenous brain distribution pattern after IV injection, strongly suggests the involvement of a direct transport of carbamazepine from nose to brain. Therefore, it seems that IN delivery represents a suitable and promising alternative route to administer carbamazepine not only for the chronically use of the drug but also in emergency conditions.

Keywords: Carbamazepine, Intranasal administration, Pharmacokinetics, Nose-to-brain drug delivery, Brain distribution, Mice

1. Introduction

Epilepsy is one of the most common and devastating neurological disorders which is estimated to have a worldwide prevalence of about 0.5-1% (White, 2003). There are several antiepileptic drugs currently available to control and suppress seizures. However, despite the ongoing development of new pharmacological therapies, more than 30% of the patients do not become seizure free mainly due to the pharmacoresistance phenomena (Weaver and Pohlmann-Eden, 2013). Moreover, conventional antiepileptic drug administration via either oral or intravenous (IV) routes commonly exhibits high systemic drug distribution into central nervous system (CNS) and non-targeted tissues which can potentiate the occurrence of drug-drug interactions and undesirable side effects that range from a CNS impairment (e.g. somnolence, dizziness and ataxia) to more severe peripheral pathological conditions such as skin reactions and hematologic, hepatic and renal dysfunctions (Toledano and Gil-Nagel, 2008).

Arguably, the delivery of drugs to the CNS remains a great challenge owing to the strict structural and functional blood brain barrier (BBB) (Gabathuler, 2010). Thus, over the last decades, different strategies have been attempted in order to circumvent the BBB and to deliver drugs efficiently into the brain for therapeutic and diagnostic applications (Gabathuler, 2010; Illum, 2000). In fact, the development of new alternative drug delivery methods could enhance the efficacy and minimize the toxicity of antiepileptic drugs, thereby improving their therapeutic index (Fisher and Ho, 2002). The intranasal (IN) administration has long been widely used for the symptomatic relief and treatment of local nasal dysfunctions, but recently, it has received a great attention as a convenient and reliable route for the systemic administration of drugs (Grassin-

Delyle et al., 2012). Nevertheless, assuming the olfactory region as a unique direct connection between the nose and the brain, an increasing interest has been posed on the potential of the IN route for the delivery of therapeutic agents directly to the CNS bypassing the BBB (Illum, 2004; Vyas et al., 2005). Indeed, IN administration represents an attractive alternative to parenteral and oral routes since, in addition to be non-invasive, it also avoids gastrointestinal and hepatic first-pass metabolism. The rapid-onset of action and the preferential delivery of drugs to the brain also enable the IN route to be successfully applied in the management of emergency situations (Li et al., 2000; Wolfe and Bernstone, 2004).

Carbamazepine (Figure 1) is one of the first-line antiepileptic drugs most commonly prescribed despite its narrow therapeutic window, complex pharmacokinetic profile, potential for drug interactions and severe side effects (Gerlach and Krajewski, 2010; Neels et al., 2004; Patsalos et al., 2008). Currently, carbamazepine is only available in tablet or suspension oral dosage forms due to its poor water solubility that prevents its incorporation in therapeutic dosages in aqueous solutions for IV injection. Following oral administration, the absorption of carbamazepine is relatively slow, erratic and formulation dependent (Landmark et al., 2012); its oral bioavailability is within the range 75-85% (Landmark et al., 2012) and the time to reach peak concentration in plasma is approximately 4-8 h post-dosing but it may be delayed by as much as 24 h with high doses (Neels et al., 2004). Furthermore, carbamazepine undergoes extensive hepatic metabolism and considerable enzymatic induction that result in unpredictable plasmatic fluctuations and unexpected clearance increments which demand successive dose adjustments (Patsalos et al., 2008; Tomson, 1987). Taking into account all those pharmacokinetic limitations of carbamazepine oral administration, we do believe that this antiepileptic drug is a promising candidate to be

administered by the IN route. A prompt and efficient IN drug delivery to the brain may decrease the systemic exposure, improving both efficacy and tolerability profiles. The opportunity to control seizures by reducing the dose makes IN administration of carbamazepine a valuable approach for long-term treatment of epilepsy. Likewise, it could also give an attractive advantage in the management of acute and severe convulsive seizure episodes. In fact, IV administration of benzodiazepines is the first-line option for the treatment of *status epilepticus* (Lockey, 2002; Manno, 2011); however, it is generally associated with hypotension, cardiac dysrhythmia and respiratory failure. Furthermore, IV injection requires sterile equipment and skilled personnel which often makes it impractical and inconvenient to use outside the hospital setting. Bearing in mind that quick cessation of the seizures is essential to prevent serious neurological damages, a rapid access and a high brain bioavailability of carbamazepine administered via IN route may probably contribute to its recognition as a viable alternative to IV administration of the drugs used in emergency conditions.

Interestingly, IN administration of carbamazepine has already been studied in rats by Barakat and collaborators (2006), reporting high levels of drug penetration in the brain solely based on the analysis of plasma and whole brain homogenates. Therefore, a comprehensive pharmacokinetic characterization of intranasal carbamazepine and its active metabolite mainly responsible for the toxic effects, carbamazepine-10,11-epoxide (Figure 1), is lacking. In this context, plasma, brain and liver levels of both carbamazepine and carbamazepine-10,11-epoxide, were, in this study, determined following IN and IV administrations to mice, and the corresponding pharmacokinetic profiles were assessed and compared. Additionally, in order to establish a more sustained basis for an hypothetic direct transport of the drug from nose to brain via the olfactory pathway, carbamazepine concentrations were also determined in different

brain regions and the rostral-caudal brain distribution of the drug was studied following the two routes of administration considered.

2. Materials and methods

2.1. Chemicals and reagents

Carbamazepine and 10,11-dihydrocarbamazepine, used as internal standard (IS), as well as Pluronic F-127 and propylene glycol were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Carbopol 974P was kindly supplied from Lubrizol (Wickliffe, OH, USA). Methanol and acetonitrile of high performance liquid chromatography (HPLC) gradient grade were acquired from Fisher Scientific (Leicestershire, UK) and Lab-Scan (Sowinskiego, Poland) respectively. Ultrapure water (HPLC grade, 18.2MΩ.cm) was prepared by means of a Milli-Q water apparatus from Millipore (Milford, MA, USA). Ethyl acetate was obtained from Fisher Scientific (Leicestershire, UK). Sodium dihydrogen phosphate dihydrate, di-sodium hydrogen phosphate dihydrate and hydrochloric acid fuming 37%, all used to prepare 0.1 M sodium phosphate buffer pH=5.0, were purchased from Merck KGaA (Darmstadt, Germany). Ketamine (Imalgene 1000®, 100 mg/ml) and xylazine (Vetaxilaze 20®, 20 mg/ml) were commercially acquired.

2.2. Animals

Adult male CD-1 mice aged between 6 and 7 weeks and weighing 30-40 g were obtained from local certified animal facilities (Faculty of Health Sciences of the University of Beira Interior, Covilhã, Portugal). Mice were housed under controlled environmental conditions (12 h light/dark cycle, at 20±2°C and relative humidity

50±5%) with free access to tap water and standard rodent diet (4RF21, Mucedola, Italy). All the experiments involving animals and their care were conducted in conformity with the international regulations of the European Directive (2010) regarding the protection of laboratory animals used for scientific purposes (2010/63/EU), and the experimental procedures employed were reviewed by the Portuguese Veterinary General Division.

2.3. Preparation of carbamazepine formulations

For IN administration, carbamazepine was previously dissolved in ethanol at the concentration of 20 mg/ml. Then 50 µl of this ethanolic solution was incorporated in 950 µl of a thermoreversible nasal gel so that the final drug concentration was 1 mg/ml and the total percentage of ethanol in the formulation was equivalent to 5%. Thermoreversible gel was prepared using the cold method described by Schmolka (1972). Briefly, 1.8 g of Pluronic F-127 (PF-127) was slowly added to 10 ml of distilled cold water (5-10°C), under gentle magnetic stirring, to achieve an efficient hydration of the flakes and then, the mixture was left at 4°C overnight to attain a complete dissolution of the polymer (18% PF-127, w/v). Afterwards, according to the technique employed by Badgujar and co-workers (2010), the mucoadhesive polymer Carbopol 974P (C-974P) was gradually dispersed in the prepared PF-127 solution with continuous agitation, until a final concentration of 0.2% w/v was reached. At this point, a nasal hydrogel formulation composed by 18% PF-127 and 0.2% C-974P was obtained, exhibiting thermo-sensible properties. In fact, PF-127 is a triblock copolymer of poly(ethylene oxide) and poly(propylene oxide) units that is fluid at or below room temperature; however it forms a gel as the temperature increases, as a consequence of the micelle packing disorder-order transition phenomenon (Swamy and Abbas, 2012).

This thermo-sensible behavior makes the final formulation suitable for gelation within the nasal cavity, providing a sustained residence of the drug at the absorption site.

For the IV administration, a carbamazepine solution was prepared as a mixture of propylene glycol-physiologic saline (0.9% NaCl)-ethanol (5:3:2, v/v/v) at a final drug concentration of 0.1 mg/ml.

2.4. IN and IV administrations

Before carbamazepine dosing, mice were always anaesthetized with an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and kept in a heated environment to maintain the body temperature.

Carbamazepine was intranasally and intravenously administered at the dose of 0.4 mg/kg. For IN administration, mice were placed on one side and 12 µl of the nasal gel per 30 g of mice body weight were instilled using a polyurethane tube (24G x 19 mm) attached to a microliter syringe. The tube was inserted about 10 mm deep into one of the nares, enabling the delivery of the formulation towards the roof of the nasal cavity. IV administration of carbamazepine (0.4 mg/kg) was performed by injection via the lateral tail vein (120 µl per 30 g body weight) using an appropriate syringe.

2.5. Pharmacokinetic studies

Mice were randomly divided into two experimental groups of 40 animals each. One of the groups received IN formulation whereas the other group was treated with the IV dosage form. At predetermined time points (5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min) after carbamazepine dosing (4 animals per time point, n = 4), the mice were sacrificed by cervical dislocation followed by decapitation and the blood was

immediately collected into heparinised tubes while brain and liver tissues were quickly removed and weighed. Blood samples were centrifuged at 4°C and 4000 rpm for 10 min to obtain plasma supernatants that were stored at -30°C until analysis. Mice brain and liver tissues were homogenized with 0.1 M sodium phosphate buffer pH 5.0 (4 ml per gram of tissue) using a THOMAS® Teflon pestle tissue homogenizer. Tissue homogenates were centrifuged at 4800 rpm for 15 min (4°C) and the resultant supernatants were also frozen at -30°C until analysis.

2.6. Brain biodistribution studies

Mice were divided at random into two experimental groups (20 animals each). The animals were treated with carbamazepine (0.4 mg/kg) using the IN or IV formulations. After administration, mice were sacrificed at 5, 10, 15, 30 and 60 min post dosing (n = 4). Blood samples were taken and plasma was separated as described above. Brains were removed and carefully dissected with the help of a scalpel into three different regions: olfactory bulb, frontal cortex and the remaining portion of the brain. The remaining portion of the brain was homogenized and centrifuged in accordance to the procedure used for brain and liver tissues, while olfactory bulb and frontal cortex specimens, regardless of the weight, were homogenized with 1 ml of phosphate buffer using an ULTRA-TURRAX® device and centrifuged at 4°C for 15 min at 13.400 rpm. The resultant homogenate supernatants were conveniently packaged and stored at -30°C until analysis.

2.7. Drug analysis

Plasma and tissue (brain and liver) concentrations of carbamazepine and carbamazepine-10,11-epoxide were determined by using a solid-phase extraction procedure followed by a reversed-phase high performance liquid chromatography (HPLC) analysis, according to the method previously developed and fully validated by Fortuna et al. (2010) with slight modifications.

Briefly, aliquots of plasma (200 µl), brain (500 µl) and liver (250 µl) homogenate supernatants were added to an appropriate volume of 0.1 M sodium phosphate buffer (pH 5.0) to make a total of 1 ml sample amount. Regarding the matrices of brain specified regions, 1 ml of both olfactory bulb and frontal cortex homogenate supernatants were used. All the samples were spiked with 10 µl of the methanolic IS working solution (200 µg/ml for all matrices excluding for the olfactory bulb, which was 100 µg/ml). After vortex mixed, samples were loaded into Waters Oasis® HLB cartridges [30 mg of hydrophilic-lipophilic-balanced (HLB) sorbent, 1 ml of capacity, from Milford, MA, USA], which were previously conditioned with 1 ml of methanol, 1 ml of acetonitrile and 1 ml of water-acetonitrile (95:5, v/v). Upon sample elution, the loaded cartridges were submitted to -30 kPa and washed four times with 1 ml of water followed by four more times with 1 ml of water-methanol (90:10, v/v). After drying the cartridge under airflow for 5 min, the drugs were eluted with 1 ml of ethyl acetate applying a gentle vacuum. The eluates were then evaporated to dryness at 45°C under moderate nitrogen stream and reconstituted with 100 µl of mobile phase by vortexing and ultrasonication. Finally, an aliquot of 20 µl (plasma, brain, liver and frontal cortex) or 40 µl (olfactory bulb) of each reconstituted extracts was injected into the chromatographic system for analysis.

The HPLC analysis was carried out on a Shimadzu liquid chromatographic system equipped with a GDU-20A_5 degasser, a SIL-20A_{HT} autosampler, a CTO-10AS_{VP}

column oven and a SPD-M20A diode array detector, all from Shimadzu Corporation (Kyoto, Japan). Data acquisition and instrumentation control were achieved by means of LCsolution software (Shimadzu Corporation, Kyoto, Japan). Chromatographic separation was performed at 40°C on a reversed-phase LiChroCART® Purospher Star® C_{18} column (55 mm x 4 mm, 3 µm; Merck KGaD), using an isocratic elution with a mobile phase consisting of water-methanol-acetonitrile (64:30:6, v/v/v) pumped at a flow rate of 1 ml/min. Carbamazepine and carbamazepine-10,11-epoxide were detected at the wavelength of 235 nm and the total running time was set at 15 min. The main partial validation parameters of the analytical method employed were in agreement with the international guidelines (FDA, 2001; EMA, 2011) and are summarized in Table 1.

2.8. Pharmacokinetic analysis

The maximum peak concentration (C_{max}) in plasma and tissues of carbamazepine and its main metabolite (carbamazepine-10,11-epoxide) and the corresponding time to reach C_{max} (t_{max}) were directly derived from the experimental data obtained. The remaining pharmacokinetic parameters were estimated based on the mean concentration values (n = 4) determined at each time point by a non-compartmental pharmacokinetic analysis employing the WinNonlin® version 5.2 (Pharsight Co, Mountain View, CA, USA). The pharmacokinetic parameters evaluated were the area under the drug concentration time-curve (AUC) from time zero to the time of the last quantifiable drug concentration (AUC_t) which was calculated by the linear trapezoidal rule; the AUC from time zero to infinite (AUC_{inf}) that was calculated from $AUC_t + (C_{last}/k_{el})$, where C_{last} is the last quantifiable concentration and k_{el} is the apparent elimination rate constant estimated by log-linear regression of the terminal segment of the concentration-time

profile; the percentage of AUC extrapolated from t_{last} to infinity [$AUC_{extrap}(\%)$], where t_{last} is the time of the C_{last}; the apparent terminal elimination half-life ($t_{1/2el}$), and the mean residence time (MRT).

The absolute bioavailability (F) of carbamazepine after IN administration was calculated as follows (Eq. 1):

$$F = \frac{(AUC_{inf\,IN} \times Dose_{IV})}{(AUC_{inf\,IV} \times Dose_{IN})} \times 100 \qquad \text{(Eq. 1)}$$

where $AUC_{inf\ IN}$ and $AUC_{inf\ IV}$ are the areas under the drug concentration-time curves from time zero to infinity following IN and IV administration, respectively; $Dose_{IV}$ and $Dose_{IN}$ are the values of the carbamazepine dosage (mg/kg) given by IV and IN route to mice.

In order to assess brain targeting efficiency of nasally delivered carbamazepine, the drug targeting efficiency (DTE) index was calculated (Wang et al., 2003). DTE index represents the brain-to-plasma partitioning ratio of the drug administered by IN route compared to that after IV injection and can be calculated according to the following equation (Eq. 2):

$$DTE = \frac{(AUC_{brain}/AUC_{plasma})_{IN}}{(AUC_{brain}/AUC_{plasma})_{IV}} \qquad \text{(Eq. 2)}$$

where AUC_{brain} and AUC_{plasma} are the areas under the drug concentration-time curves for brain and plasma after both IN and IV administration to mice. It is assured that preferential transport of drug to the brain occurs when DTE index is greater than 1 (Wang et al., 2003).

With the aim of evaluating the distribution of carbamazepine to specific brain regions (olfactory bulb, frontal cortex and the remaining portion of the brain) after its IN and IV administration, the drug concentrations in each specimen were determined at predefined time points (n = 4). The corresponding tissue-to-plasma and tissue-to-

remaining portion of the brain carbamazepine concentration ratios were calculated and compared.

2.9. Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons between IN and IV administration groups were performed using unpaired two-tailed Student's *t*-test. Differences were considered statistically significant for a *p*-value lower than 0.05 ($p < 0.05$).

3. Results

3.1. Pharmacokinetics of carbamazepine after IN and IV administration

The mean plasma, brain and liver concentration-time profiles of carbamazepine and carbamazepine-10,11-epoxide obtained in mice after a single dose of the carbamazepine (0.4 mg/kg) administered as nasal gel and IV solution are depicted in Figure 2. The corresponding main pharmacokinetic parameters estimated by non-compartmental analysis are summarized in Table 2. It is noteworthy that, in all the three biological matrices, the pharmacokinetic profiles obtained after IN and IV administration are fairly comparable. As expected, the C_{max} of the parent drug (carbamazepine) was attained almost instantaneously (5 min) after IV administration, and it occurred not only in plasma but also in brain and liver tissues. In comparison to IV delivery, only a slight delay in the time to reach the C_{max} of carbamazepine (t_{max} = 10 min) was observed for IN administration. Particularly interesting is the resemblance found in the magnitude of the peak concentrations of carbamazepine achieved in brain and plasma via IN and IV delivery. After reaching the C_{max}, carbamazepine

concentrations in plasma, brain and liver decreased similarly following the two administration routes. As shown in Table 2, the extent of systemic and brain exposure to carbamazepine was also comparable after either IN or IV administration (as assessed by AUC_t and AUC_{inf}), whereas the extent of hepatic exposure to carbamazepine was 1.4-fold greater after IV injection (as assessed by AUC_t). Thus, the absolute bioavailability estimated for carbamazepine delivered via the IN route was found to be very high (107.64%), indicating that a comparable amount of the drug was easily and rapidly accessible in the systemic circulation following both IN and IV administrations. Regarding the MRT parameter presented in Table 2, it can be noted that higher values were attained for plasma and brain after IN administration comparatively to IV administration, in contrast with the liver, where the highest MRT value was assigned to the IV route. The DTE index calculated for IN delivery of carbamazepine was 0.98 which did not provide any discriminative information of the potential for direct nose-to-brain transport of the drug via IN route. In opposition, the estimated DTE value appears to suggest that the uptake of carbamazepine into the CNS through the nasal cavity is predominately achieved by crossing the BBB after a quick nasal absorption of the drug to the systemic blood. Therefore, taking into account these pharmacokinetic data, the impact of the direct nose-to-brain delivery of carbamazepine after IN instillation was not evident when considering only the analysis of whole brain homogenate concentrations.

The concentrations of carbamazepine-10,11-epoxide were also simultaneously determined in the referred matrices. Overall, the carbamazepine-10,11-epoxide levels were near or below the limit of quantification of the analytical method, thus the estimation of the corresponding pharmacokinetic parameters was limited and therefore their values are not very informative (Table 2).

3.2. Brain biodistribution of carbamazepine after IN and IV administration

To achieve more specific and informative data on the rostral-caudal brain biodistribution of carbamazepine following its IN and IV administration (0.4 mg/kg) to mice, some particular brain regions (olfactory bulb, frontal cortex and the remaining portion of the brain) were analysed as well as the plasma samples taken at the corresponding sampling time points. The mean concentrations of carbamazepine in plasma, olfactory bulb, frontal cortex and the remaining portion of the brain up to 60 min post-dosing are presented in Figure 3. Accordingly, carbamazepine concentrations attained in plasma and in the different brain regions after IV administration of carbamazepine solution were very similar, assuming a homogenous brain distribution pattern. In contrast, following IN administration of carbamazepine nasal gel, different drug concentrations were observed throughout the specific brain regions analysed. Indeed, at 10 min post-dosing, higher carbamazepine concentrations were determined in the olfactory bulb (3.16 ± 0.09 µg/g) and frontal cortex (3.05 ± 0.09 µg/g) homogenates comparatively to the remaining portion of the brain (2.58 ± 0.09 µg/g), showing an uneven distribution of the drug from rostral to more caudal brain areas (Figure 3). Interestingly, this heterogeneous brain distribution of carbamazepine is more evident during the first three time points (5, 10 and 15 min) after the IN instillation, whereas a more uniform diffusion was accomplished from the 30 min onwards. In fact, it is noteworthy that, up to the 15 min, the highest concentrations of carbamazepine after IN administration were always found in the olfactory bulb in comparison to plasma, frontal cortex and remaining portion of the brain, sustaining a direct passage of the drug from nose to the brain.

The tissue-to-plasma and tissue-to-remaining portion of the brain concentration ratios were calculated for the olfactory bulb and frontal cortex specimens following both routes of administration (Table 3). After IV injection, similar ratios were observed at all sampling time points within the first hour post dosing, while after IN administration, discrepant values were ascertained, mainly up to 15 min. These results support the hypothesis that a direct transfer of carbamazepine from nose to the brain may be involved. Focusing particularly on the olfactory bulb-to-remaining portion of the brain ratios, it can be inferred that a direct nose-to-brain transport of carbamazepine occurs and probably via the olfactory pathway since the value of 1.29 ± 0.05 found at 5 min after IN delivery is significantly higher ($p < 0.05$) than that achieved after IV injection (0.95 ± 0.07) (Table 3).

4. Discussion

It is estimated that more than 98% of all small molecules and nearly 100% of large molecular weight drugs systemically delivered to the CNS, either by oral or IV routes, do not readily cross the BBB and reach the brain parenchyma at pharmacologically active concentrations (Pardridge, 2005). As a consequence, many promising therapeutic agents may have been discarded due to its inability to effectively permeate BBB and others are given at high systemic doses to attain therapeutic levels at the biophase, which commonly lead to undesirable peripheral adverse effects and drug interactions.

In the light of the current knowledge, drug transport across the nasal mucosa into the CNS depends on a variety of factors that can range from the physicochemical properties of the drug to the formulation design and physiological conditions at the

absorption site (Pires et al., 2009; Vyas et al., 2006). Aware that nasal mucociliary clearance is one of the major limitations for nasal drug delivery (Marttin et al., 1998), the choice of a convenient nasal dosage form that avoids the rapid nasal drainage and promotes the increase of drug residence time within the nasal cavity is fundamental (Majithiya et al., 2006). Therefore, in order to avoid a fast mucociliary clearance of the drug but simultaneously keeping an easy administration form, a thermoreversible mucoadhesive gel composed by 18% Pluronic F-127 and 0.2% Carbopol 974P was herein selected to incorporate and deliver carbamazepine by the IN route since, according to the results reported by Badgujar et al. (2010), the viscous properties of this formulation offer an appropriate and promising compromise between *in situ* gelling and ease of administration. Being a liquid-like solution at room temperature but changing to a firm gel at the physiological temperature within the nasal cavity (32-35°C) (Badgujar et al., 2010), *in situ* thermoreversible mucoadhesive gel displays a huge advantage over the conventional and more viscous hydrogels (Barakat et al., 2006; Czapp et al., 2008) concerning not only the ease of handling but also the accuracy of dosing (Basu and Bandyopadhyay, 2010).

Although carbamazepine is only currently available in oral dosage forms, it seems that the use of the IV route as a control is the most appropriate for this study. Indeed, due to the direct delivery of the drugs to the systemic circulation, IV administration will be responsible for the highest systemic exposure by comparison with any other route, creating appropriate conditions to allow a less variable drug incorporation and biodistribution. Moreover, considering that after IN administration drugs reach the CNS either via systemic circulation or olfactory epithelium, the contribution of the blood-mediated drug delivery to the brain can be inferred by employing IV injection and, consequently, the fraction of the drug directly transported from nose to brain could be more accurately discriminated.

The pharmacokinetic results herein described revealed that, similarly to what happens following IV injection, the IN administration of carbamazepine nasal gel brought a rapid and extensive systemic absorption of the drug (assessed by C_{max}, t_{max}, AUC_t and AUC_{inf}). The high carbamazepine concentrations attained in plasma after IN instillation, as well as the almost parallel time course of plasma and brain concentrations, clearly indicate that a substantial fraction of the drug has effectively been absorbed to the systemic circulation and reached the brain parenchymal tissue by crossing the BBB. In addition, comparable parent drug plasma concentration-time profiles following IN and IV administrations were also observed, supporting a similar bioavailability value (107.64%) achieved for the IN delivery of carbamazepine. These findings could be explained on the basis of the high lipophilic nature of the drug which log P value is 2.45. Indeed, small lipophilic molecules nasally administered can be rapidly absorbed to the blood stream by easily crossing the nasal membrane via transcellular diffusion and then enter into the brain after traversing the BBB. Experimental data reported in other research studies using both low molecular weight and lipophilic compounds such as diazepam (log P = 2.8) (Kaur and Kim, 2008), phenobarbital (log P = 1.47) (Czapp et al., 2008), NXX-066 (log P = 4.35) (Dahlin and Björk, 2001), progesterone (log P = 4.03) and estradiol (log P = 3.51) (van den Berg et al., 2004) underscored the fact that IN drug delivery occurred predominantly via the systemic pathway. The higher MRT values observed for plasma and brain on one hand and the lower MRT value attained in liver after IN administration comparatively to IV injection on the other hand, could also underlie the high bioavailability achieved for carbamazepine delivered by the IN route (Table 2). In fact, according to these results, the carbamazepine molecules stayed for a longer time in plasma and brain after IN

instillation in comparison with the IV injection, which in turn led to a greater retention of the drug in the liver.

Apart from the indirect pathway via the systemic circulation, it is believed that, there are two other different pathways by which a drug administered through the IN route may reach the CNS: the olfactory and the trigeminal neuronal routes (Dhuria et al., 2010). Although both of them provide a direct nose-to-brain delivery of the drug, the uptake via the olfactory neurons affords a preferential drug delivery to the olfactory bulb and rostral portion of the brain while the transference via the trigeminal nerve generally yields a more distant drug distribution to caudal brain areas. Thus, aiming at evaluating whether a direct transport of carbamazepine was occurring from the nose to the brain, the drug distribution in different brain regions was characterized after IN and IV administration. Interestingly, distinct distribution of carbamazepine through plasma, olfactory bulb, frontal cortex and remaining portion of the brain following IN and IV administration were herein reported for the first time. While a homogeneous brain distribution was observed for carbamazepine after IV injection, in the case of IN administration, the carbamazepine concentrations were different according to the respective brain area, presenting higher values in the rostral portion comparatively to the cerebral caudal region. Given that the carbamazepine brain concentration ratios determined at 5 min were 1.36-fold higher in the olfactory bulb and 1.22-fold higher in the frontal cortex employing the nasal delivery route than those obtained for IV injection (Table 3), it seems probable that a direct transport of the drug from nose to brain may be involved and that it occurred preferentially via the olfactory neuronal pathway. These findings assume particular interest in the field of the pharmacoresistant epilepsy. Indeed, it is nowadays scientifically accepted that the over-expression and/or up-regulation of multidrug efflux transporters in the BBB is one of the main

mechanisms responsible for the development of resistance to the antiepileptic drugs (Kwan et al., 2011; Löscher and Potschka, 2002; Remy and Beck, 2006). Overall, these transmembrane proteins pump the antiepileptic drugs back to the systemic circulation, restricting their access to the brain (Löscher and Potschka, 2002; Luna-Tortós et al., 2008). In this context, the results herein obtained, demonstrate that the IN route may be considered as a novel approach to overcome the pharmacoresistance phenomena since a direct delivery of carbamazepine from nose to brain was clearly evidenced and it occurred in a considerable extent.

Pooling the data derived from the pharmacokinetic and brain biodistribution studies following IN administration, it seems that with the high plasma concentrations on one hand and the superior delivery to the rostral regions of the brain on the other hand, carbamazepine reached the CNS through a combination of routes. Even though it is not possible to accurately quantify the contribution of each of these routes, we presume that a small fraction of the drug is in fact delivered to the brain via the olfactory pathway, while the most representative amount is still attributable to the systemic circulation. The 0.98 value obtained for the DTE index also strengthens this hypothesis. Notwithstanding, a further optimization of the carbamazepine nasal formulation will probably contribute to a better exploitation of the maximum potential that the IN route has to offer.

In summary, IN delivery seems to represent a suitable and promising alternative route for the carbamazepine administration regarding not only its use on the chronic treatment of epilepsy but also in the case of more severe and acute emergency situations, such as *status epilepticus.* Indeed, the IN administration of carbamazepine allowed extensive plasma and brain exposures to the drug as well as a fast and pronounced drug uptake in the brain. Apart from being very practical and adequate to be

used outside the hospital setting, the uneven biodistribution pattern with the highest CBZ concentration levels attained in the rostral areas of the brain, strengthens the potential of IN delivery to be employed in acute convulsive emergencies.

From the pharmacokinetic point of view, IN and IV administration of carbamazepine exhibited similar concentration-time profiles which probably point out to very similar pharmacological responses. In order to foresee whether IN delivery of carbamazepine could became clinically relevant, technological optimization of the nasal drug formulation, as well as further pre-clinical investigations are needed to evaluate the therapeutic efficacy attained via this route.

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Conflict of interest

All authors declare that they have no conflicts of interest concerning this work.

References

Barakat, N.S., Omar, S.A., Ahmed, A.A., 2006. Carbamazepine uptake into rat brain following intra-olfactory transport. J. Pharm. Pharmacol. 58, 63-72.

Basu, S., Bandyopadhyay, A.K., 2010. Development and characterization of mucoadhesive in situ nasal gel of midazolam prepared with Ficus carica mucilage. A.A.P.S. PharmSciTech. 11, 1223-1231.

Badgujar, S.D., Sontakke, M.A., Narute, D.R., Karmarkar, R.R., Tupkar, S.V., Barhate, S.D., 2010. Formulation and evaluation of sumatriptan succinate nasal in-situ gel using fulvic acid as novel permeation enhancer. International Journal of Pharmaceutical. Research and Development. 2, 39-52.

Czapp, M., Bankstahl, J.P., Zibell, G., Potschka, H., 2008. Brain penetration and anticonvulsant efficacy of intranasal phenobarbital in rats. Epilepsia. 49, 1142-1150.

Dahlin, M., Björk, E., 2001. Nasal administration of a physostigmine analogue (NXX-066) for Alzheimer's disease to rats. Int. J. Pharm. 212, 267-274.

Dhuria, S.V., Hanson, L.R., Frey 2nd, W.H., 2010. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J. Pharm. Sci. 99, 1654-1673.

European Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union. 276, 33-79.

European Medicines Agency, 2011. Guideline on bioanalytical method validation. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf; 2011.

Fisher, R.S., Ho, J., 2002. Potential new methods for antiepileptic drug delivery. CNS Drugs. 16, 579-593.

Fortuna, A., Sousa, J., Alves, G., Falcão, A., Soares-da-Silva, P., 2010. Development and validation of an HPLC-UV method for the simultaneous quantification of carbamazepine, oxcarbazepine, eslicarbazepine acetate and their main metabolites in human plasma. Anal. Bioanal. Chem. 397, 1605-1615.

Gabathuler, R., 2010. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. Neurobiol. Dis. 37, 48-57.

Gerlach, A.C., Krajewski, J.L., 2010. Antiepileptic Drug Discovery and Development: What Have We Learned and Where Are We Going? Pharmaceuticals. 3, 2884-2899.

Grassin-Delyle, S., Buenestado, A., Naline, E., Faisy, C., Blouquit-Laye, S., Couderc, L.J., Le Guen, M., Fischler, M., Devillier, P., 2012. Intranasal drug delivery: an efficient and non-invasive route for systemic administration: focus on opioids. Pharmacol. Ther. 134, 366-379.

Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11, 1-18.

Illum, L., 2004. Is nose-to-brain transport of drugs in man a reality? J. Pharm. Pharmacol. 56, 3-17.

Kaur, P., Kim, K., 2008. Pharmacokinetics and brain uptake of diazepam after intravenous and intranasal administration in rats and rabbits. Int. J. Pharm. 364, 27-35.

Kwan, P., Schachter, S.C., Brodie, M.J., 2011. Drug-resistant epilepsy. N. Engl. J. Med. 365, 919-926.

Landmark, J.C., Johannessen, S.I., Tomson, T., 2012. Host factors affecting antiepileptic drug delivery-pharmacokinetic variability. Adv. Drug. Deliv. Rev. 64, 896-910.

Li, L., Gorukanti, S., Choi, Y.M., Kim, K.H., 2000. Rapid-onset intranasal delivery of anticonvulsants: pharmacokinetic and pharmacodynamic evaluation in rabbits. Int. J. Pharm. 199, 65-76.

Lockey, A.S., 2002. Emergency department drug therapy for status epilepticus in adults. Emerg. Med. J. 19, 96-100.

Löscher, W., Potschka, H., 2002. Role of multidrug transporters in pharmacoresistance to antiepileptic drugs. J. Pharmacol. Exp. Ther. 301, 7-14.

Luna-Tortós, C., Fedrowitz, M., Löscher, W., 2008. Several major antiepileptic drugs are substrates for human P-glycoprotein. Neuropharmacology. 55, 1364-1375.

Majithiya, R.J., Ghosh, P.K., Umrethia, M.L., Murthy, R.S., 2006. Thermoreversible-mucoadhesive gel for nasal delivery of sumatriptan. A.A.P.S. PharmSciTech. 7(Article 67), E1-E7.

Manno, E.M., 2011. Status Epilepticus: Current Treatment Strategies. The Neurohospitalist. 1, 23-31.

Marttin, E., Schipper, N.G., Verhoef, J.C., Merkus, F.W., 1998. Nasal mucociliary clearance as a factor in nasal drug delivery. Adv. Drug. Deliv. Rev. 29, 13-38.

Neels, H.M., Sierens, A.C., Naelaerts, K., Scharpé, S.L., Hatfield, G.M., Lambert, W.E., 2004. Therapeutic drug monitoring of old and newer anti-epileptic drugs. Clin. Chem. Lab. Med. 42, 1228-1255.

Pardridge, W.M., 2005. The blood-brain barrier: bottleneck in brain drug development. NeuroRx. 2, 3-14.

Patsalos, P.N., Berry, D.J., Bourgeois, B.F., Cloyd, J.C., Glauser, T.A., Johannessen, S.I., Leppik, I.E., Tomson, T., Perucca, E., 2008. Antiepileptic drugs - best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia. 49, 1239-1276.

Pires, A., Fortuna, A., Alves, G., Falcão, A., 2009. Intranasal drug delivery: how, why and what for? J. Pharm. Pharm. Sci. 12, 288-311.

Remy, S., Beck, H., 2006. Molecular and cellular mechanisms of pharmacoresistance in epilepsy. Brain. 129, 18-35.

Schmolka, I.R., 1972. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. J. Biomed. Mater. Res. 6, 571-582.

Swamy, N.G.N., Abbas Z., 2012. Mucoadhesive in situ gels as nasal drug delivery systems: an overview. Asian J. Pharm. Sci. 7, 168-180.

Toledano, R., Gil-Nagel, A., 2008. Adverse effects of antiepileptic drugs. Semin. Neurol. 28, 317-327.

Tomson, T., 1987. Clinical pharmacokinetics of carbamazepine. Cephalalgia. 7, 219-223.

US Food and Drug Administration, 2001. Guidance for Industry: Bioanalytical method validation. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf;2001

van den Berg, M.P., Verhoef, J.C., Romeijn, S.G., Merkus, F.W., 2004. Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats. Eur. J. Pharm. Biopharm. 58, 131-135.

Vyas, T.K., Shahiwala, A., Marathe, S., Misra, A., 2005. Intranasal drug delivery for brain targeting. Curr. Drug. Deliv. 2, 165-175.

Vyas, T.K., Tiwari, S.B., Amiji, M.M., 2006. Formulation and physiological factors influencing CNS delivery upon intranasal administration. Crit. Rev. Ther. Drug Carrier Syst. 23, 319-347.

Wang, F., Jiang, X., Lu, W., 2003. Profiles of methotrexate in blood and CSF following intranasal and intravenous administration to rats. Int. J. Pharm. 263, 1-7.

Weaver, D.F., Pohlmann-Eden, B., 2013. Pharmacoresistant epilepsy: unmet needs in solving the puzzle(s). Epilepsia. 54(Suppl. S2), 80-85.

White, H.S., 2003. Preclinical development of antiepileptic drugs: past, present, and future directions. Epilepsia. 44(Suppl. 7), 2-8.

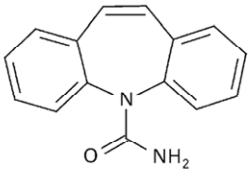
Wolfe, T.R., Bernstone, T., 2004. Intranasal drug delivery: an alternative to intravenous administration in selected emergency cases. J. Emerg. Nurs. 30, 141-147.

Figure captions

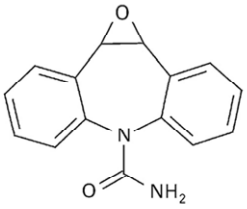
Figure 1 – Chemical structures of carbamazepine (CBZ) and its main active metabolite, carbamazepine-10,11-epoxide (CBZ-E).

Figure 2 – Concentration-time profiles of carbamazepine (CBZ) and carbamazepine-10,11-epoxide (CBZ-E) in (A) plasma, (B) brain and (C) liver tissues following intranasal thermoreversible gel and intravenous solution administration of carbamazepine (0.4 mg/kg) to mice. Symbols represent the mean values ± SEM of four determinations per time point (n = 4).

Figure 3 – Carbamazepine concentrations (mean ± SEM) up to 60 min post-dosing in plasma and different brain regions (olfactory bulb, frontal cortex and the remaining portion of the brain) after intranasal thermoreversible gel and intravenous solution administration of carbamazepine (0.4 mg/kg) to mice (n = 4, at each time point).



(CBZ)



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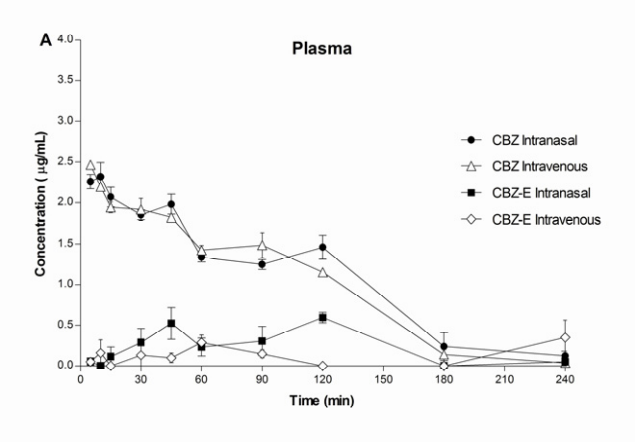

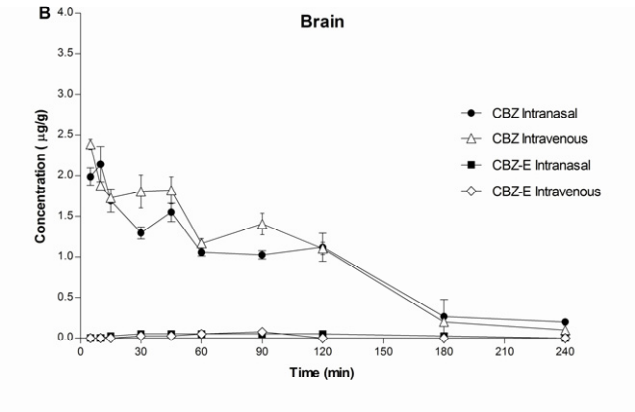

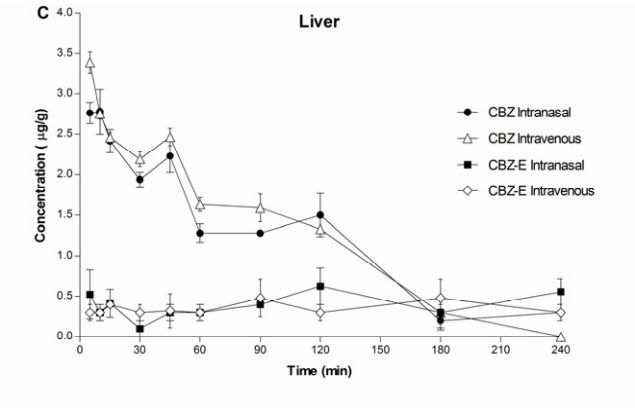

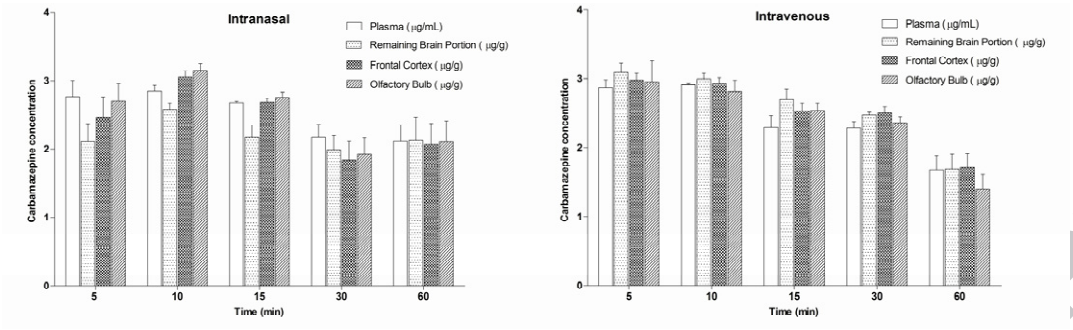

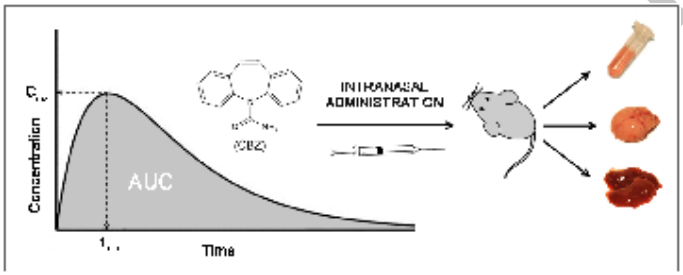

Table 1 – Validation parameters of the HPLC method employed for the quantification of carbamazepine (CBZ) and carbamazepine-10,11-epoxide (CBZ-E) in plasma, brain, liver, olfactory bulb and frontal cortex homogenate supernatants (n = 3).

Drug	Validation parameters	Plasma	Brain	Liver	Olfactory Bulb[b]	Frontal Cortex[b]
CBZ	Calibration range (µg/mL)	0.1-30	0.1-15	0.2-20	0.02-4	0.05-7.5
	Coefficient of determination (r^2)	0.999	0.997	0.999	0.998	0.997
	LOQ (µg/mL)	0.1	0.1	0.2	0.02	0.05
	Precision (%CV)[a]	≤ 6.67	≤ 7.89	≤ 3.92	≤ 5.39	≤ 7.89
	Accuracy (%Bias)[a]	-0.66-2.25	-5.41-3.75	0.28-1.55	-1.28-5.98	-5.41-3.75
CBZ-E	Calibration range (µg/mL)	0.4-30	0.05-15	0.2-20	-	-
	Coefficient of determination (r^2)	0.999	0.996	0.999	-	-
	LOQ (µg/mL)	0.4	0.05	0.2	-	-
	Precision (%CV)[a]	≤ 5.08	≤ 4.48	≤ 5.82	-	-
	Accuracy (%Bias)[a]	0.54-5.24	-6.04-4.34	-3.41-9.18	-	-

[a] Inter-day values, n = 3; [b] Calibration range and LOQ are expressed in µg; Bias, deviation from nominal value; CV, Coefficient of variation; LOQ, Limit of quantification.

Table 2 – Pharmacokinetic parameters of carbamazepine (CBZ) and carbamazepine-10,11-epoxide (CBZ-E) following the administration of carbamazepine (0.4 mg/kg) to mice through intranasal (IN) thermoreversible gel and intravenous (IV) solution.

Drug	Pharmacokinetic parameters[a]	Plasma		Brain		Liver	
		IN	IV	IN	IV	IN	IV
CBZ	t_{max} (min)	10.0	5.0	10.0	5.0	10.0	5.0
	C_{max} (µg/mL)	2.32	2.47	2.14*	2.39*	2.78*	3.39*
	AUC_t (µg.min/mL)	252.58	238.65	193.19[#]	185.71[#]	204.15[#]	288.88[#]
	AUC_{inf} (µg.min/mL)	262.46	243.84	220.58[#]	NC	NC	304.33[#]
	AUC_{extrap} (%)	3.76	2.13	12.4	NC	NC	5.07
	k_{el} (min^{-1})	0.013	0.027	0.010	0.006	0.006	0.019
	$t_{1/2el}$ (min)	55.2	25.3	70.8	127.1	112.1	35.7
	MRT (min)	76.1	64.9	71.0	52.9	53.1	64.2
	F (%)[b]	107.64	-	-	-	-	-
CBZ-E	t_{max} (min)	120.0	NA	NA	NA	120.0	90.0
	C_{max} (µg/mL)	0.60	NA	NA	NA	0.63*	0.48*
	AUC_t (µg.min/mL)	45.99	NA	NA	NA	122.87[#]	50.40[#]
	AUC_{inf} (µg.min/mL)	NC	NC	NC	NC	NC	NC
	AUC_{extrap} (%)	NC	NC	NC	NC	NC	NC
	k_{el} (min^{-1})	NC	NC	NC	NC	NC	NC
	$t_{1/2el}$ (min)	NC	NC	NC	NC	NC	NC
	MRT (min)	NC	NC	NC	NC	NC	NC

[a] Parameters were estimated using the mean concentration-time profiles obtained from four different animals per time point (n = 4). [b] Absolute intranasal bioavailability (F) was calculated based on AUC_{inf} values; * Values expressed in µg/g; # Values expressed in µg.min/g; AUC_{extrap}, Extrapolated area under the drug concentration time-curve; AUC_{inf}, Area under the concentration time-curve from time zero to infinite; AUC_t, Area under the concentration time-curve from time zero to the last quantifiable drug concentration; C_{max}, Maximum peak concentration; k_{el}, Apparent elimination rate constant; MRT, Mean residence time; NA, not available; NC, not calculated; $t_{1/2el}$, Apparent terminal elimination half-life; t_{max}, Time to achieve the maximum peak concentration.

Table 3 – Tissue-to-plasma and tissue-to-remaining portion of the brain concentration ratios of carbamazepine in different brain regions following intranasal and intravenous administration to mice (0.4 mg/kg).

Concentration Ratios	Intranasal		Intravenous							
	Post-dosing time		Post-dosing time							
	5 min	10 min	15 min	30 min	60 min	5 min	10 min	15 min	30 min	60 min
Remaining	0.76 ±	0.91	0.82 ±	0.90	0.99	1.08	1.03	1.19	1.08	1.01

Brain Portion / Plasma	0.04*	± 0.06	0.07*	± 0.03*	± 0.07	± 0.02	± 0.03	± 0.07	± 0.02	± 0.02
Frontal Cortex / Plasma	0.88 ± 0.05*	1.08 ± 0.04	1.01 ± 0.03	0.83 ± 0.05*	0.97 ± 0.06	1.04 ± 0.02	1.01 ± 0.03	1.11 ± 0.08	1.09 ± 0.02	1.03 ± 0.02
Olfactory Bulb / Plasma	0.98 ± 0.04	1.11 ± 0.03	1.03 ± 0.04	0.87 ± 0.04*	0.99 ± 0.06	1.03 ± 0.09	0.97 ± 0.06	1.12 ± 0.11	1.04 ± 0.05	0.84 ± 0.08
Frontal Cortex / Remaining Brain Portion	1.17 ± 0.05*	1.19 ± 0.05*	1.25 ± 0.09*	0.92 ± 0.03	0.98 ± 0.02	0.96 ± 0.02	0.98 ± 0.02	0.94 ± 0.05	1.01 ± 0.03	1.02 ± 0.02
Olfactory Bulb / Remaining Brain Portion	1.29 ± 0.05*	1.23 ± 0.07*	1.28 ± 0.06*	0.96 ± 0.02	1.00 ± 0.03	0.95 ± 0.07	0.94 ± 0.04	0.94 ± 0.07	0.96 ± 0.03	0.83 ± 0.08

Data are expressed as the mean values ± standard error of the mean (SEM) of four animal determinations (n = 4). Statistically significant differences ($p < 0.05$) between the two routes of administration (intranasal *versus* intravenous) are marked with an asterisk (*).