European Journal of Pharmaceutical Sciences 60 (2014) 32–39

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

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

Ana Serralheiro [a,b], Gilberto Alves [b,c,*], Ana Fortuna [a,b], Amílcar Falcão [a,b]

[a] Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal
[b] CNC – Centre for Neuroscience and Cell Biology, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal
[c] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form 23 April 2014
Accepted 28 April 2014
Available online 9 May 2014

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

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.

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

* Corresponding author. Address: Faculty of Health Sciences, University of Beira Interior, CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal. Tel.: +351 275 329002; fax: +351 275 329099.
E-mail address: gilberto@fcsaude.ubi.pt (G. Alves).

http://dx.doi.org/10.1016/j.ejps.2014.04.019
0928-0987/

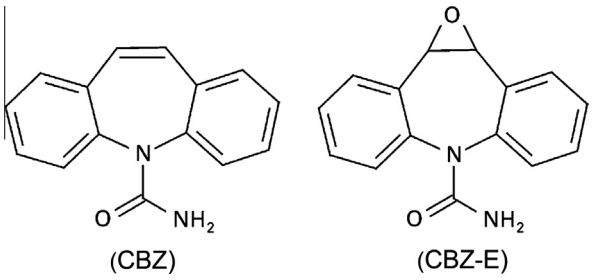

Fig. 1. Chemical structures of carbamazepine (CBZ) and its main active metabolite, carbamazepine-10,11-epoxide (CBZ-E).

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 (Fig. 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 et al. (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 (Fig. 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, 18.2 MΩ 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, disodium 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 et al. (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 thermosensible 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