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Carbamazepine uptake into rat brain following intra-olfactory transport

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Abstract

Targeting the brain via nasal administration of drugs has been studied frequently over the last few years. In this study, a suitable gel formulation was designed to provide the absorption of a highly lipophilic drug through nasal mucosa. For this purpose, carbamazepine was chosen as the model drug. Hypromellose and Carbopol were used as mucoadhesive polymers in the formulation to increase the residence time of the gel on the mucosa. The objective of this study was to confirm the existence of a transport pathway for a drug (carbamazepine) to the brain directly from the nasal cavity, by comparing the concentration of drug in the brain after intranasal (i.n.), intravenous (i.v.), and oral (p.o.) administration. A statistically significant high level of the drug was found in the brain following intranasal administration compared with the intravenous and oral routes. These findings suggested the existence of a direct transport pathway for carbamazepine from the nasal cavity to the brain. This pathway may represent a new delivery route to the brain and central nervous system of such drugs which are needed in high and rapid concentration in the brain, especially in emergencies.

Introduction

In the past decade, the use of the nasal cavity as a route for drug delivery has been an area of great interest to the pharmaceutical industry. Intranasal drug administration offers rapid absorption to the systemic blood avoiding first-pass metabolism in the gut wall and the liver. This route of administration has been shown to present a safe and acceptable alternative to parenteral administration of various drugs (Sam et al 1995; Hussain 1998). Targeting the brain via nasal administration of drugs has been studied frequently in recent years. Pardridge (1985, 1986) reviewed the strategy for the delivery of peptides to the brain and suggested that intranasal administration was a possible route. Several studies have shown a direct route of transport from the olfactory region to the central nervous system (CNS) in animal models without prior absorption to the circulating blood (Eriksson et al 1999; Dahlin et al 2000; Chow et al 2001; Illum 2004). The olfactory receptor cells are in contact with the nasal cavity and the CNS (Uraih & Maronpot 1990), and they provide a route of entry to the brain that circumvents the blood–brain barrier (BBB). This neuronal connection constitutes a direct pathway to the brain, the drugs reaching the brain parenchyma mainly by a paracellular route (Illum 2000). There are many therapeutic situations where rapid and/or specific targeting of drugs to the brain would be beneficial, such as for the treatment of Parkinson's disease, Alzheimer's disease or pain. Thus, efforts should be given to the development of a nasal delivery system capable of increasing the fraction of the drug that will reach the CNS after nasal delivery.

Nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery. However, mucoadhesive preparations have been developed to increase the contact time between the dosage form and mucosal layers of nasal cavities, thus enhancing drug absorption and preventing rapid nasal clearance (Edman et al 1992). Gels can be used for nasal drug delivery because they give a high drug transport often provided by the longer residence time of the formulation at the site of absorption (Ugwoke et al 1999). Semi-synthetic polymers such as cellulose derivatives and synthetic polymer such as Carbopol 974 P are a class of polymers, some of which can be used in nasal mucoadhesive drug delivery formulation. Their widespread use in the formulation of different types of dosage forms makes them

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quite attractive. Hypromellose, hydroxypropylcelluose and carmellose sodium have all been investigated for nasal drug administration (Vidgren et al 1992; Nakamura et al 1996).

Carbamazepine is an antiepileptic drug effective for many types of seizures, especially psychomotor seizures (Macdonald 1989). Carbamazepine belongs to class II of the biopharmaceutical classification system. Compounds in this category have low water solubility and high permeability. Consequently, the bioavailability of such compounds is limited by their solubility in water. Differences in bioavailability have been observed among various commercial formulations of carbamazepine as well as among the different polymorphic forms of the drug. Carbamazepine is absorbed slowly and erratically after oral administration (Bertilsson & Tomson 1986). Peak concentration in plasma is usually attained 4–8 h after oral ingestion but may be delayed by as much as 24 h. Reportedly, it has an oral bioavailability of less than 50% (Rall & Schleifer 1985). The efficacy of a drug can be theoretically evaluated by its concentration at the active target site as well as its blood level. The therapeutic action of an antiepileptic drug can be partially evaluated by its distribution pattern in the brain; therefore, the relationship between the distribution of carbamazepine in the brain and its anticonvulsive effect should be valuable information in the understanding of the mechanism of carbamazepine (Wang et al 2000).

The main objective of this study was to investigate the levels of carbamazepine in blood and in brain tissue samples in rats, and to find out whether the drug was transferred along the olfactory pathway to the brain following nasal administration. The uptake of carbamazepine into the brain after oral and intravenous administration was compared. It was assumed that if the brain concentration of carbamazepine was higher after nasal administration, a direct pathway from the nasal olfactory area into the brain must exist for this drug.

Materials and Methods

Materials

Carbamazepine was donated by Novartis, Carbopol 947P NF was a gift from B. F Goodrich (Cleveland, OH, USA). Phenacetin as internal standard was acquired from BDH Chemicals, Ltd (Poole, UK). Hypromellose (methocel K 4 M, nominal viscosity of a 2% aqueous solution at 20°C was 4000 cp) was purchased from Colorcon Ltd (Orpington, Kent, UK). Tween 80, propylene glycol and phosphoric acid were purchased from Sigma (St Louis, MO, USA). Acetonitrile and methanol of HPLC grade were purchased from Fisher Scientific, USA.

Preparation of carbamazepine gel

The stock (2%) gel was prepared by dispersing weighed amounts of hypromellose and Carbopol 974P (3:1) powder in distilled water in a beaker with continuous magnetic stirring. The gel was adjusted to 50 g with distilled water. To neutralize the gel to pH 7.4, 2 M NaOH was added. Carbamazepine dissolved in the least amount of alcohol was added to the formed gel. Each 50 mg gel contained 50 μg carbamazepine.

In-vitro release studies

The mucus secretion from a healthy mucosal layer has been reported to be slightly acidic and have weak ionic strength (Chien & Chang 1987). The dissolution medium of the study simulated to some extent the physical properties of the mucus of nasal cavities. The in-vitro release study was carried out with a rotating paddle using a dissolution vessel (Hansson Research, Chatsworth, CA, USA). A sample of gel (1 g) was placed in an aluminum cup (internal dimensions: 3.6 cm diameter, 0.5 cm depth). The cup was then covered with a membrane. Release medium (500 mL) was phosphate buffer, pH 6.0, maintained at 37 ± 0.5°C. The paddle was rotated at 50 rev min^{-1}. Samples (3 mL) were withdrawn at different time intervals up to 2 h and replaced with fresh buffer dissolution medium. Samples were filtered through a membrane filter (0.45 μm) and carbamazepine concentration was analysed spectrophotometrically at 285 nm.

Data analysis

Korsmeyer at al (1983) derived a single relationship which described drug release from polymeric drug delivery systems.

This equation can be used to analyse the first 60% of a release curve where the release is linearly related to t^n, regardless of the geometric shape:

$$M_t/M_\alpha = kt^n \quad (1)$$

where M_t/M_α is the fraction of drug released at time t, k (with units t^{-n}) is a constant incorporating the properties of the polymer and the drug, and n is the diffusional exponent characteristic of the release mechanism. For example, with swellable spherical matrices, $n = 0.45$ for Fickian diffusion, $0.45 < n < 0.85$ for anomalous (non-Fickian) transport, and $n > 0.85$ for case II transport (Ritger & Peppas 1987).

Animal studies

The animal studies were conducted in accordance with the guide of The Animal Research Committee of King Saud University in Riyadh, Saudi Arabia. A total of 81 male Sprague-Dawley rats were used (Mollegaard, Denmark), with a mean weight of 250 g (range 200–300 g). The animals were housed in the animal breeding facility of the laboratory for 4–6 days during the acclimation period and during the course of the study. They were housed in standard cages, in a light-controlled room (10:14 h dark/light cycle) and temperature-controlled environment (20 ± 2°C and 50 ± 5% r.h.). Food and water were freely available. Food was withdrawn 24 h before the experiments. Water was freely available during fasting and throughout the experiment. In this study, rats remained conscious throughout the experiment, thereby providing functional mucociliary transport throughout the procedure. This was ideal for the testing of putative mucoadhesive gel formulations.

In-vivo absorption studies

Intranasal administration

Carbamazepine was prepared in 2% gel preparations containing hypromellose:Carbopol 974P, in a ratio 3:1. Carbamazepine concentration was 50 μg/50 mg gel. For intranasal administration, 50 mg (0.2 mg kg^{-1}) was injected via a silastic tubing (o.d.: 1.19 mm, i.d.:0.64 mm) injected 2 cm into one of the nares towards the roof of the nasal cavity. Each rat was held in a supine position and received the intranasal dose. The rats were held in this supine position for 1 min after administration. After application the actual dose received was assessed by visual inspection of the pipette tip. It was estimated that 80% of the dose was accepted when administered by the rat nostril only.

Intravenous administration

The intravenous injection (8 mg kg^{-1}) was given as a bolus of carbamazepine solution in propylene glycol–pure water–ethanol (5:3:2; v/v/v, 10 mg mL^{-1}). Carbamazepine was injected into the tail vein using a catheter.

Oral administration

Via the therapeutic oral dose 4 mg drug was administered to rats. Carbamazepine was suspended in 3% Tween 80, 20% propylene glycol and 77% physiological saline (16 mg mL^{-1}) prepared 1 h before use and administered orally via gastric intubations (16 mg kg^{-1}) to each rat.

The animal was decapitated and blood samples were collected into heparinized centrifuge tubes just before dosing and at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min during the study. Blood samples were centrifuged at 4000 rev min^{-1} for 10 min in a Hermlez centrifuge (Germany). Plasma was separated and immediately stored at −20°C until analysed for carbamazepine. The blood collection was generally completed within 15 s. Following the completion of blood collection, the skull of the animal was opened and the whole brain was collected. The collection of the whole brain was typically completed within 1 min following the blood collection. The brain samples were quickly rinsed with saline and blotted dry with filter paper to get rid of blood-taint and macroscopic blood vessels as much as possible. After weighing, the brain tissue samples were homogenized with 1 vol saline in a tissue homogenizer (Ika-Ultra-Turrax DI 25, Germany), and immediately stored at –20°C until analysis. The sample collection process always involved two laboratory personnel: one responsible for dosing and sample collection and the other for tracking the time and sample handling. Measurements were made using three rats at each time point.

Assay of carbamazepine in plasma and brain samples

Carbamazepine was assayed using an HPLC method (Kobayashi et al 2000). An approximately 0.5-mL sample of plasma or brain homogenate was mixed with 50 μL of the internal standard solution, phenacetin (10 μg mL^{-1}), and the mixture was extracted with 6 mL ethyl acetate. After 5 min centrifugation (3500 rev min^{-1}), the samples were frozen at –28°C, and the organic layer was collected and evaporated under vacuum at 45°C. The drug residue was reconstituted in 100 μL mobile phase and injected into the HPLC system for analysis. Concentration of carbamazepine in the samples was determined using an HPLC apparatus (Shimadzu SCL-10A VP, Japan) equipped with a 15 cm × 4 mm i.d. VP-ODS C_{18} column (Shimadzu, 5 μm, Japan), C_{18} precolumn and Shimadzu SPD-10A VP-UV detector. Calibration curves of carbamazepine were prepared with plasma and brain tissue mixed with known amounts of the drug, utilizing its HPLC peak area ratios to the internal standard. The mean best fit linear regression equation was used to estimate the concentrations of carbamazepine at different time intervals. The conditions of the HPLC method were as follows: mobile phase consisted of 50:50 methanol:water; flow rate was set at 1.3 mL min^{-1}; volume of the injected sample was 20 μL; detector wavelength set at 285 nm. Under these conditions the retention times were 5.8 min for carbamazepine and 3.4 min for phenacetin.

Assay validation

The mean calibration curve for carbamazepine ($y = 13724.2x - 1631.7$) (n = 6) was linear within the concentration range between 0.24 and 15 µg mL^{-1} (or μg g^{-1}) ($r^2 = 0.9976$). The mean extraction recovery of carbamazepine from plasma or brain tissue homogenate was more than 90.4 ± 5.7% and 94.5 ± 7.2%, respectively. The intra-assay coefficients of variation (CV) for the standard of the calibration curve (n = 4) ranged from 2.3 to 7.1%. The values for the inter-assay CV for the same concentrations (n = 6) were between 1.2 and 5.3%. The inter- and intraday accuracy (expressed as the percentage error of the determined concentration compared with the theoretical concentration) was lower than 4.6 and 9.5%, respectively. The percentage plasma or brain recovery of carbamazepine and internal standard as well as the inter- and intra-assay CV were confirmed to the requirements for validation (Shah et al 1992).

Pharmacokinetics analysis and statistics

All concentration data were dose and weight-normalized. Pharmacokinetic parameters were estimated using model-independent methods (Gibaldi & Perrier 1982). RSTRIPE (RSTRIP ver. 5 micromath Scientific Software, Salt Lake City, UT, USA), a curve stripping, and PCNONLIN (PCNONLIN ver. 4 Statistical Consultant, Apex, NC, USA), nonlinear least squares regression computer programs were utilized to estimate the pharmacokinetic parameters of carbamazepine. The non-compartmental model was adapted for calculating the pharmacokinetic parameters. The parameters used to evaluate were the maximum plasma or brain tissue concentration (C_{max}) and the corresponding time (t_{max}) which was read directly from the individual plasma or brain concentration against time profiles. The area under the plasma or brain concentration–time curve ($AUC_{0-2\,h}$) and the area under the first moment curve (AUMC) were estimated by the linear trapezoidal rule and extrapolated to infinity using standard techniques. The mean residence time (MRT) was calculated as $AUMC/AUC_{0-\infty}$.

The bioavailability (F) was calculated according to the following equation:

$$F = (AUC_{i.n.\ p.o.} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{i.n.\ p.o.}) \quad (2)$$

where $AUC_{i.n.\ p.o.}$ and $AUC_{i.v.}$ denote the means of individual AUC values from the intranasal, oral and intravenous groups, respectively. The statistics were assessed using the Kruskal–Wallis test. Individual differences between the three routes of carbamazepine administration were determined using a non-parametric post hoc test and a value of $P < 0.05$ was considered statistically significant. Results are presented as mean values ± s.d.

Results and Discussion

In-vitro release of carbamazepine

Using equation 1, a good relationship between log M_t/M_α and log t was found, with r^2 greater than 0.966. The release of carbamazepine from the gel preparation followed a non-Fickian release mechanism, $n = 0.643$, suggesting an anomalous transport of carbamazepine in the gel. This indicated that not only the diffusion process of carbamazepine but also the swelling of the polymers controlled the release of carbamazepine from the gel. The results were in agreement with the release of diclofenac sodium from gel prepared using poloxamer 407 and hypromellose (Pongjanyakul et al 2005). The time taken for release of 50% of the drug (t50%) was 48 min.

In-vivo absorption and brain distribution of carbamazepine

The BBB presents a challenge for the treatment of several CNS disorders, since many drugs are not able to effectively transverse the vascular endothelium to reach their target in the parenchyma. It is therefore thought that treatment of these diseases could be improved if drug delivery across the BBB could be increased. Additionally, there is marked variability in patient response to drugs that have been in use for epilepsy for several years. It is well established that a formidable membranous barrier severely hampers the access of many drugs into the brain and central nervous system. Three elements underlie the BBB function: a physical barrier comprised of tight junctions, which form a tight seal to intercellular diffusion; the cells themselves, which exhibit a low rate of endocytosis; and a metabolic barrier, consisting of specific membrane transports expressed by endothelial cells (Lai et al 2005; Maines et al 2005).

The olfactory route of drug administration provides a route of entry to the brain that circumvents the BBB, and this neuronal connection constitutes a direct pathway to the brain.

Rats have been used widely for nasal drug delivery studies. Rats are classified as macrosomatic i.e. the olfactory epithelium occupies a large area (50% for the rat) (Gross et al 1982) of the total nasal epithelium. However, man is classified as microsomatic with a total olfactory area of approximately 3% (Morrison & Costanzo 1990). Further, in man, the olfactory region is located in the roof of the cavity, while in rats the olfactory area is spread throughout the whole cavity, as it is in many other common animal models. It is important to take these anatomical differences between species under consideration when results are interpreted and compared.

Figures 1–3 show the mean plasma and brain concentration–time profiles of carbamazepine following intranasal, intravenous and oral administration to rats at a dose of 0.2, 8 or 16 mg kg^{-1}, respectively. The initial absorption of carbamazepine from the gel formulation into the brain tissue after nasal administration was rapid (Figure 1). High brain carbamazepine concentration was attained following intranasal administration. This was followed by a gradual decline in brain drug concentration and in a few cases a plateau of brain drug levels was obtained. In some cases a mucoadhesive formulation may not always sustain nasal drug absorption. Ugwoke et al (2000) showed rapid release of apomorphine from degradable starch microspheres, despite its proven mucoadhesive properties.

Nasal absorption of carbamazepine from a gel preparation into the systemic circulation exhibited the retardant release effect and the maximum plasma concentration (2.3 ± 0.7 μg mL^{-1}) was achieved at 45 min (Figure 1). The profiles of carbamazepine level in brain tissue displayed an initial absorption phase and maximum concentration (12.5 ± 1.3 μg g^{-1}) after approximately 5 min. Following intravenous administration (Figure 2), brain carbamazepine

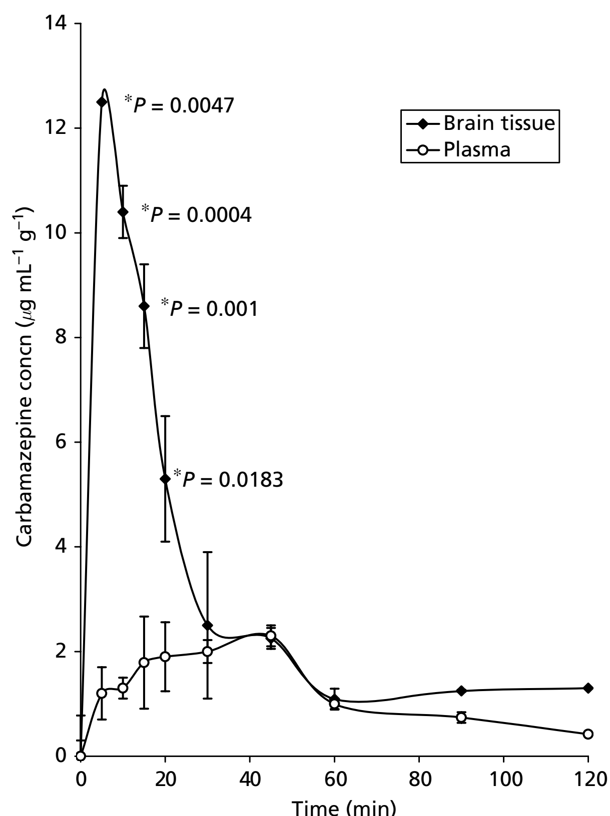

Figure 1 Plasma and brain tissue concentration–time profiles of carbamazepine 0.2 mg kg^{-1} following intranasal administration in rats. Each point represents the average of three rats. *Significant difference ($P < 0.05$) between carbamazepine levels in brain and plasma at each data point.

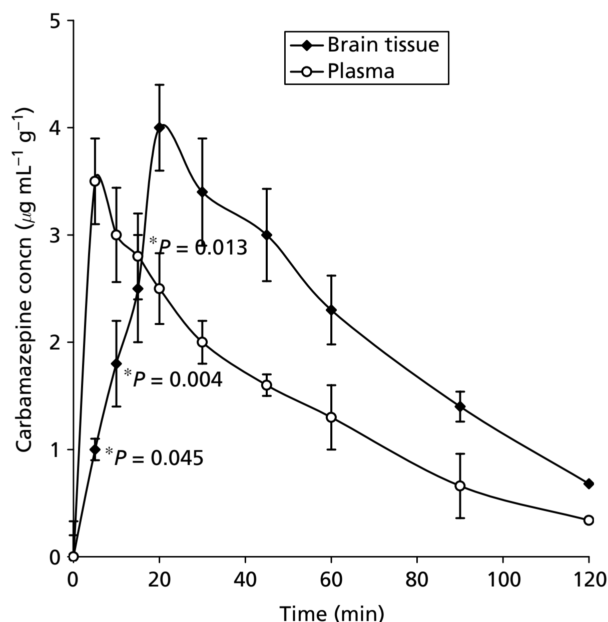

Figure 2 Plasma and brain tissue concentration–time profiles of carbamazepine 8.0 mg kg^{-1} following intravenous administration in rats. Each point represents the average of three rats. The initial intravenous concentrations were obtained by extrapolation. *Significant difference ($P < 0.05$) between carbamazepine levels in brain and plasma at each data point.

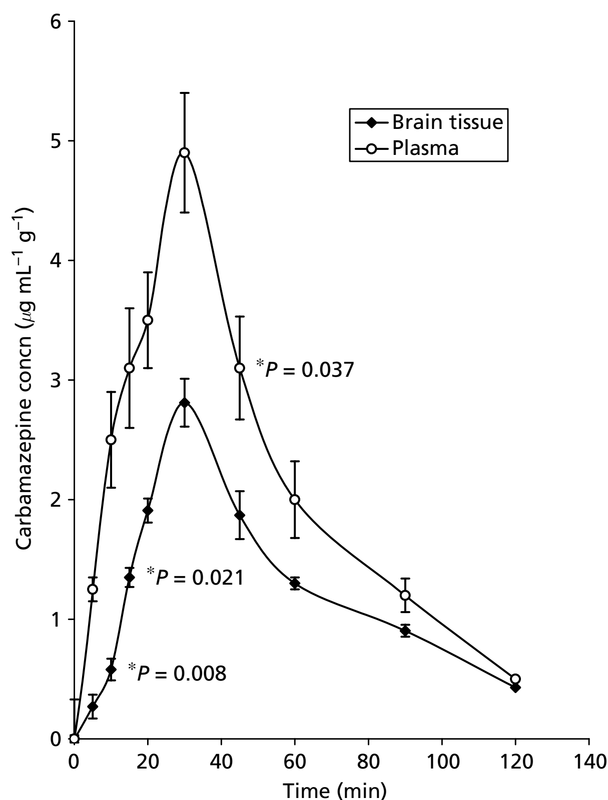

Figure 3 Plasma and brain tissue concentration–time profiles of carbamazepine 16.0 mg kg^{-1} following oral administration in rats. Each point represents the average of thee rats. *Significant difference ($P < 0.05$) between carbamazepine levels in brain and plasma at each data point.

reached a peak level ($4.0 \pm 1.5\,\mu g\ g^{-1}$) at 20 min as a result of the high initial plasma carbamazepine concentration after the bolus intravenous administration, which caused high, rapid transport of carbamazepine crossing the BBB by passive diffusion (Figure 2). On the other hand, brain carbamazepine concentration reached maximum level ($2.8 \pm 0.9\,\mu g\ g^{-1}$) at 30 min following oral administration (Figure 3). This meant that a 3- and 4.5-fold increase respectively in C_{max} was achieved in brain tissue following intranasal administration compared with intravenous and oral administration. Concentration of carbamazepine in plasma increased gradually after intranasal administration and reached maximum after 45 min. This may have been the result of delayed absorption of carbamazepine across the nasal membrane into the systemic circulation. Perhaps because of its high lipophilicity carbamazepine was accumulated in the mucosa and then slowly delivered into the blood.

Figure 4 illustrates the brain-to-plasma carbamazepine concentration ratios following intravenous, intranasal and oral administration as a function of time. The brain tissue-to-plasma ratios of carbamazepine following intranasal administration were found to be significantly higher than those following intravenous and oral administration. At 5 min post dosing, the brain-to-plasma carbamazepine concentration ratios were found to be 10.4 ± 2.4, 0.42 ± 0.08 and 0.22 ± 0.03, following intranasal, intravenous and oral administration, respectively. Irrespective of the dose differences, statistically significant differences were found between these ratios ($P < 0.05$). The rapid appearance of carbamazepine in the brain tissue illustrated the utility in nasal drug delivery that could be exploited in administering emergency drugs. After 30 min, the brain tissue-to-plasma carbamazepine concentration

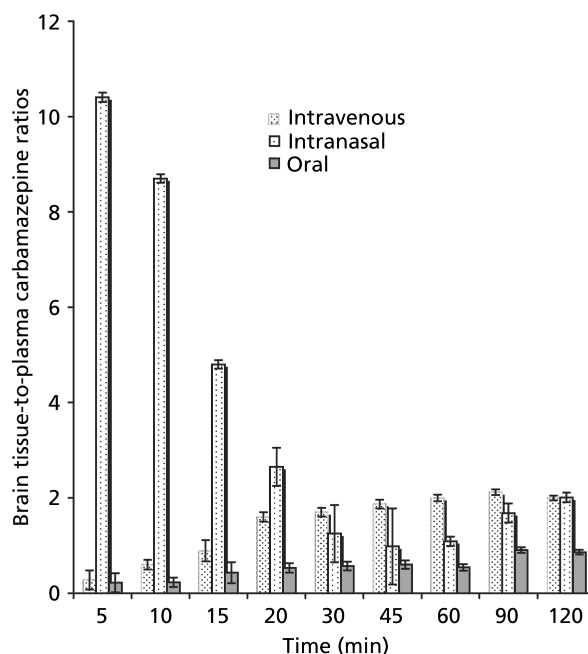

Figure 4 Brain tissue-to-plasma carbamazepine concentration ratios as a function of time following intranasal gel (0.2 mg kg^{-1}), intravenous solution (8 mg kg^{-1}), and oral suspension (16 mg kg^{-1}) administration in rats.

ratio reached a plateau following intranasal administration, the ratio then increased again at 90 min. The brain tissue-to-plasma carbamazepine concentration ratio following oral and intravenous administration was less than 1 and gradually increased with time. This suggested the rapid existence of carbamazepine in plasma rather than in brain tissue i.e. systemic absorption of carbamazepine preceded carbamazepine brain distribution when using the oral route of administration.

It is believed that drug uptake into brain from the nasal mucosa is via two different pathways. One is the systemic pathway, where some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing the BBB. The other is the olfactory pathway, whereby drug can travel from the olfactory region in the nasal cavity directly into cerebrospinal fluid (CSF) and brain tissue (Illum 2000). We could deduce that the amount of the drug in the brain tissue after nasal application was attributed to these two pathways.

Concentration of carbamazepine in the brain was found to be much higher than that in the plasma following intranasal administration, showing that there existed a direct transport pathway from the nose to the brain and that transport was comparatively rapid. These findings were in good agreement with Ikada et al (1992) for the intranasal administration of dopamine to rats and supported the existence of a nose–brain direct pathway. Kumar et al (1974, 1982) showed that progesterone and estradiol, which are lipid soluble, achieved higher levels in CSF after intranasal than after intravenous administration.

Intra-olfactory delivery of insulin resulted in a significant absorption of the hormone into the brain as early as 10–15 min after the instillation (Gizurarson et al 1997). Our finding was consistent with previous reported results. In our study, the existence of a direct transport pathway of carbamazepine was confirmed also. Carbamazepine achieved remarkably higher levels in the brain tissue after nasal administration as compared with intravenous or oral administration, although the intranasal dose was the smallest dose administered to rats ($0.2\,mg\,kg^{-1}$). It has been demonstrated that the direct pathway from the nasal cavity into the CNS is quite selective. Kumar et al (1979) reported that peak drug levels in the CSF after intranasal administration of progesterone were not only much higher than those obtained after intramuscular administration, but that the T_{max} in the CSF was significantly shorter than that in the serum. In contrast, the T_{max} for norethisterone did not precede the serum T_{max} following nasal spray administration. The elimination of norethisterone from the CSF was also significantly slower compared with progesterone. Hydroxyzine, a piperazine-derivative antihistamine, showed preferential absorption into the CSF after being instilled into the nasal cavity, while chlorcyclizine, another member of the piperazine family, could only be detected in the plasma, not in the CSF (Chou & Donovan 1997). The dissimilar distribution patterns between the structurally similar compounds indicated that there was significant selectivity based on chemical properties or structure for transport between the nasal cavity and the CSF. Lipid soluble substances with a molecular weight less than 600 Da may readily permeate the BBB depending on their partitioning coefficients. Lipophilicity and molecular weight have long been believed to be major determinants of the transfer of drugs into CNS across the BBB (Pardridge 1991). A series of studies by Sakane et al (1991, 1994, 1995) showed a relationship between transport from the nasal cavity to the CSF and lipophilicity, dissociation properties and molecular weight, respectively. The nose–brain pathway as a conduit for transmission of an agent into the CNS is an area of ongoing research. A number of substances, approximately 35–40 including viruses, metals, dyes and drugs, have been determined to gain direct access to the CSF and/or brain after nasal administration (Mathison et al 1998). However, this does not appear to be the case for (*S*)-5-fluoro-8-hydroxy-2-(dipropyl-amino) tetralin ((S)-UH-301), a serotonin-1A receptor antagonist. It showed no increased uptake into the CSF after nasal administration compared with intravenous administration, i.e. there was no evidence that (S)-UH-301 was transported from the nasal cavity along the olfactory neuron into the brain, although the physicochemical characteristics, such as molecular size and partition coefficient, allowed (S)-UH-301 to be transported rapidly across most membranes (Dahlin & Björk 2000). Those results were similar to those from another study with a cognition enhancer (Hussain et al 1990). Hussain et al (1990) suggested that the nose–brain pathway might only be significant for poorly absorbed substances. For substances that were rapidly and completely absorbed into the systemic circulation, any transport along the olfactory neurons into the CNS was slow and insignificant. A good correlation exists between the lipid solubility of a drug and its ability to penetrate or diffuse across the BBB (Oldendorf 1974). However, a physostigmine analogue is a lipophilic compound with a significant tissue binding, which could explain the low concentration of the drug in the CSF (Dahlin & Björk 2001).

To decide if a substance has been transported into the CNS, it is important to determine the concentration at several time points. In a study with zidovudine (Seki et al 1994), CSF concentration in rats was measured once, 15 min after administration. The concentration of zidovudine in the CSF was not significantly higher following nasal administration than after intravenous infusion, and no direct nose–brain pathway was proven. However, if several CSF or brain samples had been taken at different time points and AUC values had been calculated, the conclusions might have been different. One major limitation of most of the previous work was that brain and CSF drug concentrations usually were determined at a single time following dosing. Depending on the time of sampling, the brain drug concentration obtained following intravenous administration could have passed the maximal level and already have been in the declining phase, while the brain drug concentration obtained following intranasal administration could have been just around the maximal level. One could then incorrectly conclude that enhanced drug exposure in the brain had been observed following intranasal administration, implying the existence of direct nose–brain drug transport.

The pharmacokinetic parameters for carbamazepine in brain tissue and plasma after intranasal, intravenous and oral administration are presented in Table 1. The absorption of carbamazepine after oral administration into the systemic circulation was slow and not complete. $AUC_{0-2\,h}$ values (plasma samples) after oral and intravenous administration were 272.6 ± 25.6 and $181.3 \pm 23.5\,\mu g\,min\,mL^{-1}$, respectively, and consequently bioavailability was 75.9%. However, $AUC_{0-2\,h}$

Table 1 Pharmacokinetic parameters[a] of carbamazepine following intranasal gel (0.2 mg kg^{-1}), intravenous solution (8 mg kg^{-1}), and oral suspension (16 mg kg^{-1}) administration in rats[a]

Parameters	Brain tissue			Plasma		
	Intranasal	Intravenous	Oral	Intranasal	Intravenous	Oral
AUC_{0-2h} (μg min mL^{-1} g)	343.8 ± 22.43[b]	265.4 ± 24.7	156.1 ± 15.8	149.3 ± 19.6	181.3 ± 23.5	272.6 ± 25.6
P value	Intranasal–intravenous > 0.05	Intravenous–oral > 0.05	Oral–intranasal < 0.05	Intranasal–intravenous > 0.05	Intravenous–oral > 0.05	Oral–intranasal < 0.05
C_{max} (μg mL^{-1} g)	12.5 ± 1.3	4.0 ± 1.5	2.8 ± 0.9	2.3 ± 0.7*	–	4.9 ± 1.1
P value	Intranasal–intravenous > 0.05	Intravenous–oral > 0.05	Oral–intranasal < 0.05			
T_{max} (min)	5.0 ± 0.9	20.0 ± 0.4	30.0 ± 1.4	45.0 ± 1.6**	–	30.0 ± 1.8
P value	Intranasal–intravenous > 0.05	Intravenous–oral > 0.05	Oral–intranasal < 0.05			
MRT (min)	31.9 ± 10.2	61.6 ± 9.4	67.4 ± 7.6	64.7 ± 9.1	43.5 ± 6.0	54.6 ± 10.1
P value	Intranasal–intravenous > 0.05	Intravenous–oral > 0.05	Oral–intranasal < 0.05	Intranasal–intravenous > 0.05	Intravenous–oral > 0.05	Oral–intranasal < 0.05

All statistical tests were conducted after dose normalization of AUC_{0-2h} and C_{max}. [a]Parameters were calculated from the average concentrations obtained, n = 3. [b]Mean ± s.d. *The two-tailed *P* value is 0.0368 (significant). **The two- tailed *P* value is 0.019 (significant).

Table 2 Mean area under curve (AUC) values from carbamazepine concentration–time profiles ($\mu g\ mL^{-1}$) in plasma and ($\mu g\ g^{-1}$) in brain tissue after intravenous solution ($8\ mg\ kg^{-1}$), intranasal gel ($0.2\ mg\ kg^{-1}$) and oral suspension ($16\ mg\ kg^{-1}$) administration to rats.

Time (min)	Intranasal		Ratio B/P[a]	Intravenous		Ratio B/P	Oral		Ratio B/P
	Brain ($\mu g\ min\ g^{-1}$)	Plasma ($\mu g\ min\ mL^{-1}$)		Brain ($\mu g\ min\ g^{-1}$)	Plasma ($\mu g\ min\ mL^{-1}$)		Brain ($\mu g\ min\ g^{-1}$)	Plasma ($\mu g\ min\ mL^{-1}$)	
0–30	209.7 ± 11.3	48.7 ± 18.5	4.31 ± 0.45	84.2 ± 12.5	84.1 ± 12.3	1.00 ± 0.38	39.5 ± 13.6	108.5 ± 11.3	0.36 ± 0.27
P value	=0.0001*			=0.6914***			=0.0001*		
30–60	60.7 ± 9.5	57.0 ± 11.5	0.94 ± 0.36	90.5 ± 19.4	51.7 ± 11.3	1.74 ± 0.24	58.7 ± 15.6	90.5 ± 20.4	0.65 ± 0.46
P value	=0.1221***			=0.0001*			=0.0012**		
60–120	73.4 ± 8.7	43.6 ± 13.5	0.96 ± 0.31	91.2 ± 17.5	45.5 ± 16.6	2.00 ± 0.14	57.9 ± 17.4	73.6 ± 15.4	0.79 ± 0.39
P value	=0.0015**			=0.0004*			=0.0127**		

[a] $B/P = AUC_{brain\ tissue}/AUC_{plasma}$. *Extremely significant; **significant; ***not significant.

(brain tissue) after oral and intravenous administration were 156.1 ± 15.8 and 265.4 ± 24.7 μg min g^{-1}, respectively, and bioavailability was 29.4%. On the other hand, the absorption of carbamazepine from the nasal cavity into the brain tissue was rapid and complete, nasal administration showed the highest value of AUC_{0-2h} compared with the intravenous and oral route of administration: 343.8±22.4, 265.4±24.7, 156.1±15.8 μg min g^{-1}, respectively ($P = 0.0036$). Statistical tests comparing C_{max}, T_{max} and MRT (brain tissue) after different routes of administration revealed significant levels ($P = 0.049$, 0.0036, 0.011, respectively). Dunn's multiple comparison test revealed a significant difference between intranasal and oral for all the pharmacokinetic parameters calculated ($P < 0.05$). As it was mentioned before, the therapeutic action of an antiepileptic drug can be partially evaluated by its distribution pattern in the brain; therefore, a high concentration of carbamazepine in the brain tissue was our goal to achieve in this study. A fairly short time to peak brain concentration (~5 min after nasal administration) had already been reached with the first sampling point in all animals receiving carbamazepine nasally. This confirmed the rapid absorption of small lipophilic compounds after nasal administration. MRT (brain tissue and plasma) was noticeably increased following intravenous and oral administration.

To allow comparison of brain carbamazepine content after different routes of administration, brain carbamazepine contents were therefore normalized by the plasma carbamazepine content of the corresponding time point and the brain-to-plasma carbamazepine AUC ratios at different time intervals; 0–30, 30–60, 60–120 min following all three routes of administration were calculated (Table 2). Results showed that the ratio of AUC_{0-30} in brain tissue to that in plasma after nasal administration was significantly higher ($P < 0.05$); the AUC ratio was 4.31 times higher (209.7 ± 11.3 vs 48.7 ± 18.5). Following intravenous administration the AUC_{0-30} ratio in brain tissue to that in plasma was similar as a result of a rapid decline of the carbamazepine level after intravenous administration and rapid uptake of carbamazepine into the brain tissue crossing the BBB. Following oral administration the AUC_{0-30} ratio was less than 1 as a result of transferring of the carbamazepine from the circulation after systemic absorption and then distribution into the brain tissue.

The direct pathways for transfer of substances from the olfactory mucosa into the CNS can be broadly classified as the olfactory nerve pathway and the olfactory epithelial pathway (Illum 2002). For very lipid soluble compounds, diffusion or retrograde axonal transport within the neuron can lead to targeting of the olfactory bulbs followed by diffusion to the rest of the brain. This route of transport is relatively slow and is probably not relevant in terms of drug administration. The second route of transport is thought to exist via the subarachnoid space, which extends along the olfactory nerve to the basolateral side of the olfactory epithelium. This route enables relatively quick absorption to the CSF of hydrophilic and semi-lipophilic substances. Further, since the CSF is not the target site for most CNS-active drugs, the compounds should able to penetrate into the brain parenchyma to exert a pharmacological response before they are drained to the circulating blood together with the CSF (Bagger & Bechgaard 2004).

Many CNS-active substances, however, are biologically active in low concentrations and encouraging findings of Throne et al (1995) and Chen et al (1998) studying wheat germ agglutinin–horseradish peroxidase (WGA-HRP) and recombinant human neuro-growth factors (NGF), respectively, showed that it was possible to achieve therapeutically active concentrations of the proteins in the brain via the olfactory pathway after nasal administration.

Conclusion

The study has demonstrated that it was possible to prepare a nasal formulation of carbamazepine which resulted in fast and pronounced absorption, with a potential for clinical application in acute situations. In conclusion, a direct transport pathway of carbamazepine to brain tissue from the nasal cavity has been confirmed. Intranasal administration may represent a valuable delivery route to brain with an appropriate dosage form design. Many CNS active substances are potent drugs but unfortunately these substances are often associated with unwanted side effects. If it was possible to administer carbamazepine nasally and utilize the nose–brain pathway, dose and side-effects could be reduced. This pathway could prove to be a useful route for CNS-active substances which do not normally pass the blood–brain barrier in sufficient amounts.

Further studies are necessary to see whether this route may be clinically relevant and to determine whether intranasal carbamazepine could be used not only in medical centres, but at home for parents of children with seizures, after appropriate instructions had been given.

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