International Journal of Pharmaceutics 375 (2009) 55–60

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Brain pharmacokinetics of tetramethylpyrazine after intranasal and intravenous administration in awake rats

Jian Feng [a], Fanzhu Li [a,*], Yanmin Zhao [a], Yaorong Feng [a], Youichi Abe [b]

[a] Department of Pharmaceutics, Zhejiang Chinese Medical University, Hangzhou 310053, PR China
[b] Department of Pharmacology, Kagawa University Medical School, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

ARTICLE INFO

Article history:
Received 29 December 2008
Received in revised form 13 March 2009
Accepted 28 March 2009
Available online 5 April 2009

Keywords:
Tetramethylpyrazine
Microdialysis
Pharmacokinetics
Intranasal administration
Rats

ABSTRACT

Brain pharmacokinetic behaviors of tetramethylpyrazine (TMP) following intranasal (i.n.) and intravenous (i.v.) administration, have been investigated using brain microdialysis technique in free-moving rats. A cross-over design was employed in the present experiment. The same set of rats ($n = 5$) received i.v. injection at a dose of 10 mg/kg TMP via tail vein. Equal dose was administered intranasally. After application, the dialysates sampled from the left striatum were measured by HPLC-UV detection. The results indicated that the mean corrected TMP concentration of 1.49 μg/ml was obtained at 5 min following i.n. dosing while no TMP in the dialysate sampled 5 min after i.v. injection was detected, in the range of our measurement limit. No compartment model was most suitable for analysis of the concentration vs. time results after i.n. dosing. Thus, a non-compartment model was used in the analysis of all experimental data. No significant differences in brain pharmacokinetic parameters, except Cmax, were found between both i.n. and i.v. administration routes. AUCi.n./AUCi.v. ratio was 92.42%. Finally, compared with i.v. application, intranasal administration of TMP could obtain significantly fast absorption from nasal to ipsilateral striatum and equal bioavailability. Therapeutically relevant nasal formulation is a potential alternative for intravenous administration approach for TMP.

1. Introduction

In natural products, TMP has been shown to have an extensive bioactivity, extracted from Chinese herbal medicine *Ligusticum wallichii* Franch in 1957. TMP is widely used in the treatment of disorders of cerebrocardiac vessel, central nervous function and cognitive system in China. For therapeutic agent of TMP, microcirculation perfusion improvement (Dai and Bache, 1985; Xue et al., 1989), capillary permeability alleviation (Tuttle et al., 1989; Xue et al., 1989), inhibiting characteristics on platelet and antithrombotic effects (Li et al., 2001, 2004; Sheu et al., 2001) have been reported mainly during the past two decades. As to its effects on central nervous system, Zhang et al. (2004) indicated that TMP attenuated the learning and memory dysfunction in D-galactose-lesioned mice through improving antioxidative and central cholinergic system function, and protecting NMDA receptor activity. The same finding was also described by Ni et al. (1995). TMP provides significant neuroprotection against ischemic brain and spinal cord injury, and relieve various neurological symptoms through its anti-inflammatory reaction, anti-apoptosis (Fan et al., 2006) and reduction of neuronal apoptosis (Liao et al., 2004; Kao et al., 2006). In hippocampal neurons, TMP prevented hypoxic and excitotoxic cell damage (Shih et al., 2002).

The protective effects of TMP on brain neuronal and cognitive function are of significant importance for its clinical application. However, TMP of oral dosing has specifical pharmacokinetic characteristics with short biological half-life of 0.5–2 h, low oral bioavailability of 10–30% (Lou et al., 1986). Invasive intravenous infusion for 4–6 h, predominant dosing approach of TMP in clinical therapy, produces maintenance pharmacodynamical effect but a poor compliance. Although transdermal delivery system has been investigated, these studies are still only focused on the percutaneous permeability (Qi et al., 2002). Thus, a more appreciable administration route of TMP to enhance brain delivery ability was required.

Intranasal administration is often considered as a safe and acceptable route of drug delivery of brain targeting, gaining significant recent interest, especially for the substances with biological effects on the CNS (Illum, 2000, 2002; Graff and Pollack, 2005). Intranasal substrates gain access to the brain via the olfactory route, circumventing the blood–brain barrier (BBB) and via peripheral circulation (Vyas et al., 2005). Considerable studies to present have been undertaken on intranasal delivery system (Illum, 2000, 2002; Shahiwala and Misra, 2004). Rapid mucociliary clearance of drugs intranasally administered is one of the two most important factors

* Corresponding author. Tel.: +86 571 8663 3030; fax: +86 571 8661 3607.
E-mail addresses: lifanzhu@zjtcm.net, lilongzhu911@sohu.com (F. Li).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.03.034

contributing to limited nasal absorption (Illum, 2003). Therefore, a design using an awake animal model in the study is also suggested to be employed due to an effect of the anaesthetic agents to a variable degree on the mucociliary clearance (Mayor and Illum, 1997), and to make the experimental data on nose–brain delivery more conformable to physiological condition.

Microdialysis sampling technique is believed to be a unique tool to assay unbound drug disposition and metabolism from extracellular fluid in different tissues that is gaining popularity in pharmacokinetic studies (de Lange et al., 2000; Tsai, 2003). Brain microdialysis applies microdialysis technique on sampling analytes in brain, which allows continuous collection of dialysates in the local brain region of interest with high spatial and temporal resolution. There are limitations associated with recovery problem and surgical trauma for microdialysis (de Lange et al., 1999; Tsai, 2003). Nevertheless, brain microdialysis has been exhibiting great advantages in brain research, including maintenance of brain physiological function, altering no CSF and brain interstitial fluid volume and analysis based on free extracellular compounds.

Although there are studies available concerning the pharmacokinetics of TMP after i.v. injection in brain and blood of anaesthesia rats, as far as we are aware the present investigation was first carried out using brain microdialysis technique in free-moving rats on brain pharmacokinetic behavior of TMP following i.n. dosing. The aim of the present study was to evaluate a preferentially alternative administration approach for TMP through a comparison of nasal and intravenous delivery.

2. Materials and methods

2.1. Chemicals

Tetramethylpyrazine hydrochloride was provided by National Institute for the Control of Pharmaceutical and Biological products (Beijing, China). Methanol was purchased from E. Merck (Darmstadt, Germany). Solvents (Millipore Corp., Bedford, MA) were of HPLC grade used for all preparations. All other chemicals were of analytical grade and commercially available.

2.2. Animals

Five male adult Sprague–Dawley rats weighing approximately 350 g were obtained from the Laboratory Animal Center of Zhejiang Chinese Medical University (Zhejiang, China). Animals were acclimatized for at least 5 days with alternating dark/light cycle of each 12 h in a climate controlled room with temperature maintained at $22 \pm 1\,^{\circ}C$ and a relative humidity of $60 \pm 10\%$. Water and standard laboratory food were available *ad libitum*. All experiments were performed according to the guidelines for the care and use of animals as established by Zhejiang Chinese Medical University.

2.3. Artificial CSF and formulations

Artificial CSF (aCSF), consisting of 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 2.0 mM K_2HPO_4, 2.0 mM KH_2PO_4 and 0.1 mM ascorbic acid, adjusted to pH 7.4, was used as perfusion medium for brain microdialysis probes (Bagger and Bechgaard, 2004).

A standard stock solution of TMP was prepared by dissolving 10.0 mg of TMP in 25 ml of aCSF for use in sample analysis. TMP dosing solutions were prepared by dissolving TMP in sterile saline for intravenous administration to a concentration of 10.0 mg/ml and in isotonic phosphate buffer (pH 7.4) for nasal administration to a concentration of 200 mg/ml, respectively. Aliquots of these formulations were kept at $4\,^{\circ}C$ until the day of the experiment.

2.4. Surgical procedures

Sprague–Dawley rats were anaesthetized with ketamine/xylazine (90 mg/kg + 10 mg/kg) by intraperitoneal injection and mounted on a stereotaxic frame (Bioanalytical Systems, West Lafayette, IN, USA). The surgical procedure was described elsewhere (Bergquist et al., 1996). Briefly, a 0.5 mm hole in a rat was drilled in the skull (+3.2 mm lateral to the midsagittal suture and 0.2 mm anterior to bregma). A chronic brain microdialysis guide cannula (MD-2251, BAS, West Lafayette, IN, USA) with stylet in place was inserted into the brain aimed to the left striatum, identically to a depth of 7.0 mm ventrally from the dura according to the Rat Brain Atlas of Paxinos and Watson (1997). The guide cannula was fastened to the cranium with skull screws and dental acrylic cement. After the surgery the animals were allowed to recover for 6 days in single cages under standard conditions (12 h light/dark cycle, a controlled ambient temperature of $22 \pm 2\,^{\circ}C$ and a relative humidity of $60 \pm 10\%$), with free access to food and water until 24 h before administration, at which time only food was withdrawn.

2.5. Recovery determination

The *in vivo* retrodialysis and *in vitro* recoveries of the drug TMP were both determined as described before (Bagger and Bechgaard, 2004). The *in vivo* retrodialysis average recovery by the loss of TMP was used for further recalculations of actual extracellular unbound drug concentrations in sampling region left striatum.

Prior to probe implantation both *in vitro* recoveries obtained by gain and loss (retrodialysis) of three probes of the same type for use in brain (MD-2200, BAS, West Lafayette, IN, USA) with characteristics of 0.5 mm O.D., 2 mm membrane length and nominal 38 kDa MW cut-off, were investigated at 2.0 μl/min flow-rate with three concentration levels (0.5, 1.0 and 5.0 μg/ml). Thus, the *in vitro* recoveries (gain and loss) for TMP were 33.6% and 38.4%, respectively, between which no significant difference ($P > 0.05$) was found. No difference between the three individual probes was shown ($P > 0.05$). The *in vitro* recoveries (gain and loss) were used only to evaluate the functional properties of each probe.

After the present cross-over pharmacokinetic experiment on each rat, the *in vivo* recovery of TMP was assessed for the brain microdialysis probe under the assurance of TMP concentration below the limit of detection posterior to a washout period. The above three concentrations of TMP solutions in aCSF were also used as perfusates at a flow-rate of 2.0 μl/min throughout the studies. Three of the samples (20 μl per sample) from each period were collected and analyzed over 10-min intervals for 60 min (first two and last samples discarded).

The *in vivo* recovery by loss of TMP for each probe at striatum was calculated from the following equation:

$$\text{Recovery}_{(\text{TMP } in\ vivo)} = \frac{C_{\text{TMP dialysate}} - C_{\text{TMP perfusate}}}{C_{\text{TMP BIF}} - C_{\text{TMP perfusate}}},$$

where C_{TMP} is the TMP concentration, given for dialysate, perfusate and BIF (brain interstitial fluid), respectively. During the retrodialysis period, the concentration of TMP in BIF was assumed to be zero due to its relative high diffusion coefficient in brain tissues. All statistical analyses (ANOVA) were performed by Origin 6.0 (Microcal Software, Inc., MA, USA).

2.6. Microdialysis experiments

A cross-over design was employed in the present experiment. In three of five animals, TMP was first dosed intranasally and later, after a 5 h of washout period, the drug was intravenously

injected. Another two rats were given the reverse drug administration. Animals received i.v. administration at a dose of 10 mg/kg TMP via tail vein. Equal dose of TMP was administered intranasally. In a supine-70 position was the rat under control using a homemade patent device when nasal formulations were dosed. Nasal preparations were administered with a soft PE-10 tubing (Becton, Dickinson and Company, USA) fitted to a 50 μl Hamilton microsyringe, inserting 15 mm into the cavity in the left nostril (Bagger and Bechgaard, 2004). All microdialysis experiments were performed in conscious freely moving animals kept in an awake animal caging system (Stand-Alone Raturn and Rodent Bowl kit, BAS, West Lafayette, IN, USA), with no anaesthesia used throughout the experiment.

On the day of the experiment a brain microdialysis probe (MD-2200) was inserted into the guide cannula, 45 min before sampling was begun. The inlet of the probe was attached to a BAS syringe driver (MD-1001, USA) connected with a controller (240 V/50 Hz MD-1000K, BAS, West Lafayette, IN, USA), filled with aCSF as perfusion fluid. The brain microdialysis probe was perfused at a 2 μl/min flow-rate for 45 min (delay time) and all microdialysates collected were discarded in order to eliminate the void volume in tubing and stabilize solute levels around the dialysis membrane before starting the collecting of samples. Posterior to this equilibrium period, brain microdialysate samples were collected for an additional 90 min automatically using refrigerated fraction collector (MD-1201, BAS, West Lafayette, IN, USA) into 300 μl vials under a 10 min sampling regime throughout the experiments (sample volume = 20 μl per vial). All samples were stored at −20 °C until analysis.

At the end of each washout period, blank dialysate samples were also collected to ensure that no TMP concentration was detected prior to the following administration or *in vivo* recovery determination.

After each experiment followed by *in vivo* recovery assessment, the animals were anaesthetized with excessive chloral hydrate and transcardially perfused with 4% paraformaldehyde in 0.1 M Sorenson buffer as described previously (Williams et al., 1995). The brains were dissected out, immediately frozen and stored at −20 °C. Coronal 40 μm thick slices were later cut in a cryo-microtome and the unstained sections were observed under magnification to localize the probe tract. Only animals with probe located in the olfactory bulb and cerebellar nuclei were included in the present study—others were not assayed.

2.7. HPLC assay method and pharmacokinetic analyses

Concentrations of TMP were determined by HPLC-UV analysis as described by Liang et al. (1999). TMP standards were prepared in aCSF solution .The HPLC system (all from Agilent Technologies, Wilmington, DE, USA) was equipped with a binary pump (G1312A), VWD detector (G1314A) with a detection wavelength of 280 nm, column (20BAX SB RP-18 5 μm) and a data system for chromatogram integration (Agilent Chemistation data analysis system). The mobile phase consisted of methanol–water (50:50, v/v), adjusted to pH 3.0 with orthophosphoric acid at a flow-rate of 1.0 ml/min. The retention of TMP was 6.0 min. The limit of detection of TMP was 0.04 μg/ml using 10 μl samples volumes, estimated as the TMP quantity corresponding to three times the baseline noise. The linear range of the analytical method was 0.1–5.0 μg/ml for TMP (r^2 = 0.9996). Intra-day and inter-day variabilities were determined at concentrations of 0.1–5.0 μg/ml using the HPLC method on the same day and 5 different days, respectively. All of the R.S.D. were less than 15% (Causon, 1997).

The most appropriate pharmacokinetic models were selected by WinNonlin 4.0.1 software (pharsight, Mountain View, CA, USA) using the concentrations vs. time data of TMP after nasal and intravenous application, respectively, based on the correlation coefficients between Y and Y predicted (r^2) and values of Akaike's information criteria (AIC) to examine the goodness of fit. The mean i.v. and i.n. areas under the curve (AUC) values were calculated using linear-trapezoidal model and AUCi.n./AUCi.v. ratios were also evaluated for two routes of administration to assess the direct brain delivery of TMP from nose. The times to peak concentrations (Tmax) were obtained directly from the plot of concentration–time data. Various pharmacokinetic parameters were determined following the model fitting and presented as mean ± S.D.

2.8. Statistical analysis

Statistical analyses (ANOVA) of mean results across multiple treatment groups were performed by Origin 6.0 (Microcal Software, Inc., MA, USA), followed by paired Student's *t*-test. A *P*-value below 0.05 was taken to indicate significant difference between data means. All values were presented as mean ± S.D.

3. Results

Average recovery by loss (retrodialysis) *in vivo* (n = 5) of four brain probes (MD-2200) with three concentration levels of TMP solutions in aCSF (0.5, 1.0 and 5.0 μg/ml), respectively, used in left striatum of four rats was 12.81% (S.D. ± 1.54%). This was used to correct the actual concentration of TMP in dialysates sampled from left striatum in rats, by the following equation: absolute concentration = dialysate concentration/recovery$_{(\text{TMP } in\ vivo)}$.

The recalculated unbound TMP in left striatum concentration–time profiles following i.n. and i.v. application, respectively, are presented in Fig. 1. Since 10 ml volume existed in the tube between the dialysis probe outlet and the fraction collector in our microdialysis experimental system, accordingly, the actual first sampling time-point was at 5 min after both routes of administration (i.v. and i.n.) under a 10 min sampling regime at 2 μl/min flow-rate. The decrease of the amount of dialysate sampled due to the decreased sampling time course may result in the resulting quantity of TMP in dialysate out of the limitation of the HPLC-UV analyses optimized. As can be found easily from Fig. 1, the mean corrected TMP concentration of 1.49 μg/ml was obtained at 5 min following i.n. dosing while no TMP in the dialysate sampled 5 min after i.v. injection was detected, in the range of our measurement limit.

Drug average concentrations plotted were all considered the same as external concentrations at the end of 10 min rather than at the mid-time point of each single collection interval. Similar to the report (Tmax = 20 min) by Tsai and Liang (2001), the TMP concentrations in left striatum both reached the maximum levels at 15 min after both dosing, respectively, whereas the mean corrected peak concentration (Cmax) following i.v. injection was higher compared to that after i.n. administration (P < 0.05). It was also seen clearly that almost identical concentration–time elimination curves following i.n. and i.v. application were found. Posterior to the Tmax, the corrected mean concentrations of TMP declined relatively sharp. And, at the last sampling time-point of the present protocol, the amount of the TMP in dialysates sampled by microdialysis in left striatum was close to the lower limit of detect for both routes of administration. The same result of our pilot study made us to select 5 h as the washout period in the cross-over protocol on free-moving rats.

The data following i.v. injection fitted well a two-compartment open-system pharmacokinetic model that was consistent with prior report (Tsai and Liang, 2001), whereas no compartment model was most suitable for analysis of the concentration vs. time results after i.n. dosing. Thus, a non-compartment model was used in the analysis of all experimental data, in order to compare

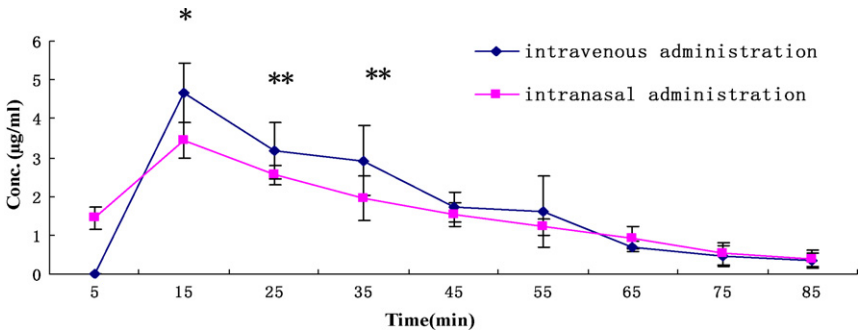

Fig. 1. Average extracellular unbound TMP concentrations in left striatum as a function of time after intranasal and intravenous administration, respectively, of 10 mg/kg TMP to rats. All data are corrected by *in vivo* recovery and represent the mean ± S.D. ($n = 5$). *Significant difference found between i.n. and i.v. application ($P < 0.05$). **Significant difference not found between i.n. and i.v. application ($P > 0.05$).

brain pharmacokinetic parameters of TMP in left striatum after i.n. dosing with those following i.v. injection. The correlations of individual fittings (r^2) between observed and predicted values of corrected TMP concentrations were in the range of 0.970–0.988. Selected pharmacokinetic parameters derived from the individual time–concentration profiles from the microdialysis experiment in conscious rats were shown in Table 1, including AUC, Cmax, MRT, Cl, Vz and $t_{1/2\,el}$. No significant differences between both routes of administration were found for all the recalculated pharmacokinetic parameters, except Cmax ($P = 0.014$). The values of significance in total body clearance (Cl) and volume of distribution based on the terminal phase (Vz) between i.v. and i.n. application, respectively, were $P = 0.614$ and $P = 0.336$. For the i.v. route, the volume of distribution at steady state (Vss) was 797.40 ± 116.77 ml. As shown in Table 1, the value of mean i.n. AUC was smaller than that of mean i.v. AUC but there is no statistical significance between both the values ($P = 0.122$). AUCi.n./AUCi.v. ratios in left striatum after i.n. and i.v. administration was 92.42%.

4. Discussion

TMP possesses an appreciable blood–brain barrier penetrability with a Tmax of 20 min in brain and a progressively high $AUC_{brain/blood}$ following intravenous injection, which has been supported by clinical CNS efficacy (Ho et al., 1989; Ni et al., 1995) and basic pharmacokinetic data (Tsai and Liang, 2001). Tsai and Liang (2001) conducted the TMP determination in dialysates using HPLC and demonstrated that the mean brain level of about 3 μg/ml of unbound TMP was obtained at the first sampling time-point of 10 min following i.v. bolus and subsequently reached to peak brain concentration of close to 4 μg/ml. Rather, 5 min after administration in the present study was employed as the first collecting time-point for drug assay to elevate temporal solution in the absorption phase. The TMP concentrations measured in the dialysate samples collected over a sampling interval were not the concentrations at the sampling time-points but rather accumulative levels of the collection interval. Thus, the recovery problem of microdialysis probes for drug of interest and the sensitivity of drug determination method make the amount of collected dialysates related to sampling intervals and flow-rate of perfusates a challenge, especially for the drugs with rapid disposition characteristics. Compared with 3 μg/ml in brain at 10 min after i.v. injection, only a trace of TMP around the lower detect limit or no TMP were found for the four individual experimental rats at 5 min after administered intravenously, whereas 1.49 μg/ml at the same time-point after i.n. application both subsequently followed by peak concentration (Cmax) at 15 min post-treatment. This interesting phenomenon observed evidently suggested that i.n. TMP could be delivered into brain via nose–brain direct pathway, at least in part, at the early absorption phase.

Table 1
Selected pharmacokinetic parameters for tetramethylpyrazine hydrochloride administered intravenously and intranasally to freely moving rats at 10 mg/kg, respectively.

Parameters	i.n.	i.v.	Ratio (i.n./i.v.)
Tmax (min)	15[b]	15[b]	
Cmax (μg/ml)	3.28 ± 0.49[a]	4.48 ± 0.11	
MRT (min)	35.29 ± 0.61	34.10 ± 0.38	
Cl (ml/min)	25.47 ± 5.40	23.41 ± 3.70	
Vz (ml)	542.23 ± 49.87	483.73 ± 78.27	
$t_{1/2\,el}$ (min)	15.08 ± 2.75	14.36 ± 1.54	
$AUC_{0-\alpha}$ (min μg/ml)	131.43 ± 4.94	142.21 ± 8.16	92.42%
Vss (ml)		797.40 ± 116.77	

Data are expressed as mean ± S.D. ($n = 5$).
[a] Significant difference ($P < 0.05$) vs. i.v. administration.
[b] Observed value.

Direct delivery of drug from nose to brain, circumventing the blood–brain barrier (BBB), has been evidenced to be a distinct possibility by considerable reports in the literatures of studies. The pathway employed for transport of drugs into brain is dependent on molecular weight (MW) and the lipophilicity (Sakane et al., 1991, 1995; Illum, 2000). Accordingly, three nose–brain pathways are proposed to be modes for a drug to reach the CNS from the nasal cavity, which are transcellular pathway, paracellular pathway and intracellular axonal transport (Illum, 2003). Of these pathways, the paracellular route by passing through the tight junctions or through open clefts in the membrane is especially suited for low molecular weight hydrophilic agents (Illum, 2003). Considering the physicochemical characterization of TMP with MW of 136.2 and the partition coefficient (Ko/w) of 123.5, it is more probable that TMP administered nasally was delivered into brain paracellularly in part. Simultaneously, some of the TMP could be absorbed from nasal mucosa into the systemic circulation where TMP could either cross the BBB and reach the brain or be eliminated via normal clearance mechanisms (Hussain et al., 1980), as described by Illum (2003). Therefore, it could be concluded prudently that the aforementioned both clearance effects resulted in the lower peak concentrations in brain for i.n. TMP than for i.v. TMP. In addition, the mucociliary clearance is also a factor of importance limiting the nasal absorption.

After intravenous bolus, two-phasic disposition of TMP in brain was observed (Tsai and Liang, 2001). This was also confirmed by the present brain pharmacokinetic results of i.v. TMP, best fitting the kinetics of a two-compartment model according to the values of correlation coefficient ($r^2 = 0.98$) and AIC. However, the disposition curves following i.n. administration of TMP may not be represented as any compartment models. We proposed in the present study,

therefore, a non-compartment model of intravenous and intranasal TMP, respectively, in order to enable the analyses of brain pharmacokinetics of TMP and comparison of TMP kinetic process in brain after both i.v. and i.n. administration.

Since a fairly high total clearance ($CL_{i.n.}$ = 25.47 ml/min) and volume of distribution (Vz = 542.23 ml) were observed, the brain elimination ($t_{1/2\ el}$ = 15.08 min) might demonstrate that some of the TMP administered nasally had been cleared in the systemic circulation and nasal mucosa or from the nasal cavity into the gastrointestinal tract before being uptaken into brain via nose–brain direct pathway and BBB penetration. The same was also displayed for pharmacokinetic parameters of i.v. TMP. No significant differences in the remaining pharmacokinetic parameters were founded between both i.n. and i.v. administration routes, including AUC. The ratio of $AUC_{i.n./i.v.}$ of 92.42% in brain also suggested that i.n. dosing TMP might obtain the same bioavailability in brain as i.v. injection TMP did.

Microdialysis sampling technique, causing minimal perturbation to physiological processes, has been to date extensively used in and greatly contributory to neurosciences, pharmacodynamics and drug disposition and metabolism researches (Davies, 1999), in which brain microdialysis study is especially of significance in that traditional brain research methods exhibit evidently more disadvantages. Microdialysis technique with exclusive characteristics of high temporal and spatial resolutions is quite suitable for drug delivery research, specifically in the brain. Additionally, brain microdialysis also makes it feasible to perform real time and on-line experiment on free-moving animals. The study by Mayor and Illum (1997) suggested that the use of anaesthetics was proposed to a variable extent to attenuate the ability of the nasal mucociliary clearance. As a result, a prolonged residence time of nasal formulation in the nasal cavity was obtained. In this paper, a free-moving rat model with no anaesthesia introduced was utilized throughout the experiment in order to deplete the influence of anaesthesia on absorption via nasal mucous membrane, with access to physiological condition. The application of microdialysis technique and awake animal model contributed to employing a cross-over experimental design, which not only reduced the amount of experimental animal used and experimental cost, but also minimized the influence of anatomical and physical differences between individual animals on experimental data.

In summary, compared with i.v. application, intranasal administration of TMP could obtain significantly fast absorption from nasal to ipsilateral striatum and equal bioavailability. Even if not completely satisfactory from a pharmacokinetical view point due to the relative low brain uptake obtained, the nasal route for administration offers particularly interesting perspectives for the *in vivo* evaluation of the action peptides on the CNS. In order to maintain therapeutic brain levels for long periods, a controlled release nasal formulate is under development in our lab. Therapeutically relevant nasal formulation of TMP is a potential alternative for intravenous administration.

Acknowledgements

This work was financially supported in part by Project No. 30371781 and No. 30772793 of the National Natural Science Foundation of China.

References

Bagger, M.A., Bechgaard, E., 2004. The potential of nasal application for delivery to the central brain—a microdialysis study of fluorescein in rats. Eur. J. Pharm. Sci. 21, 235–242.

Bergquist, J., Vona, M.J., Stiller, C.O., O'Connor, W.T., Falkenberg, T., Ekman, R., 1996. Capillary electrophoresis with laser-induced fluorescence detection: a sensitive method for monitoring extracellular concentrations of amino acids in the periaqueductal grey matter. J. Neurosci. Methods 65, 33–42.

Causon, R., 1997. Validation of chromatographic methods in biomedical analysis viewpoint and discussion. J. Chromatogr. B: Biomed. Sci. Appl. 689, 175–180.

Dai, X.Z., Bache, R.J., 1985. Coronary and systemic haemodynamic effects of tetramethylpyrazine in the dog. J. Cardiovasc. Pharmacol. 7, 841–849.

Davies, M.I., 1999. A review of microdialysis sampling for pharmacokinetic applications. Anal. Chim. Acta 379, 227–249.

de Lange, E.C., de Boer, A.G., Breimer, D.D., 1999. Microdialysis for pharmacokinetic analysis of drug transport to the brain. Adv. Drug Deliv. Rev. 36, 211–227.

de Lange, E.C., de Boer, A.G., Breimer, D.D., 2000. Methodological issues in microdialysis sampling for pharmacokinetic studies. Adv. Drug Deliv. Rev. 45, 125–148.

Fan, L.H., Wang, K.Z., Cheng, B., Wang, C.S., Dang, X.Q., 2006. Anti-apoptotic and neuroprotective effects of tetramethylpyrazine following spinal cord ischemia in rabbits. BMC Neurosci. 14, 7–48.

Graff, C.L., Pollack, G.M., 2005. Nasal drug administration: potential for targeted central nervous system delivery. J. Pharm. Sci. 94, 1187–1195.

Ho, W.K., Wen, H.L., Lee, C.M., 1989. Tetramethylpyrazine for treatment of experimentally induced stroke in Mongolian gerbils. Stroke 20, 96–99.

Hussain, A., Foster, T., Hirai, S., Kashihara, T., Batenhorst, R., Jones, M., 1980. Nasal absorption of propranolol in humans. J. Pharm. Sci. 69, 1240.

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

Illum, L., 2002. Nasal drug delivery: new developments and strategies. Drug Discov. Today 7, 1184–1189.

Illum, L., 2003. Nasal drug delivery—possibilities, problems and solutions. J. Control. Release 87, 187–198.

Kao, T.K., Ou, Y.C., Kuo, J.S., Chen, W.Y., Liao, S.L., Wu, C.W., Chen, C.J., Ling, N.N., Zhang, Y.H., Peng, W.H., 2006. Neuroprotection by tetramethylpyrazine against ischemic brain injury in rats. Neurochem. Int. 48, 166–176.

Liang, C.C., Hong, C.Y., Chen, C.F., Tsai, T.H., 1999. Measurement and pharmacokinetic study of tetramethylpyrazine in rat blood and its regional brain tissue by high-performance liquid chromatography. J. Chromatogr. B: Biomed. Sci. Appl. 724, 303–309.

Liao, S.L., Kao, T.K., Chen, W.Y., Lin, Y.S., Chen, S.Y., Raung, S.L., Wu, C.W., Lu, H.C., Chen, C.J., 2004. Tetramethylpyrazine reduces ischemic brain injury in rats. Neurosci. Lett. 372, 40–45.

Li, M., Handa, S., Ikeda, Y., Goto, S., 2001. Specific inhibiting characteristics of tetramethylpyrazine, one of the active ingredients of the Chinese herbal medicine 'Chuanxiong,' on platelet thrombus formation under high shear rates. Thromb. Res. 104, 15–28.

Li, M., Zhao, C., Wong, R.N., Goto, S., Wang, Z., Liao, F., 2004. Inhibition of shear-induced platelet aggregation in rat by tetramethylpyrazine and salvianolic acid B. Clin. Hemorheol. Microcirc. 31, 97–103.

Lou, Y.Q., Zhang, H., Cao, X., Chen, M.L., 1986. The pharmacokinetics and disposition of tetramethylpyrazine phosphate in dogs and rats. Acta Pharm. Sin. 21, 481–487 (in Chinese with English abstract).

Mayor, S.H., Illum, L., 1997. Investigation of the effect of anaesthesia on nasal absorption of insulin in rats. Int. J. Pharm. 149, 123–129.

Ni, J.W., Matsumoto, K., Watanabe, H., 1995. Tetramethylpyrazine improves spatial cognitive impairment induced by permanent occlusion of bilateral common carotid arteries or scopolamine in rats. Jpn. J. Pharmacol. 67, 137–141.

Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coordinates, 3rd ed. Academic Press, Sydney.

Qi, X., Ackermann, C., Sun, D., Liu, R., Sheng, M., Hou, H., 2002. The prediction of plasma and brain levels of 2,3,5,6-tetramethylpyrazine following transdermal application. AAPS PharmSci. 4, E46, http://www.aapspharmsci.org.

Sakane, T., Akizuki, M., Taki, Y., Yamashita, S., Sezaki, H., Nadai, T., 1995. Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the molecular weight of drugs. J. Pharm. Pharmacol. 47, 379–381.

Sakane, T., Akizuki, M., Yamashita, S., Nadai, T., Hashida, M., Sezaki, H., 1991. The transport of a drug to the cerebrospinal fluid directly from the nasal cavity: the relation to the lipophilicity of the drug. Chem. Pharm. Bull. (Tokyo) 39, 2456–2458.

Shahiwala, A., Misra, A., 2004. Nasal delivery of levonorgestrel for contraception: an experimental study in rats. Fertil. Steril. 81 (Suppl. 1), 893–898.

Sheu, J.R., Hsiao, G., Lee, Y.M., Yen, M.H., 2001. Antithrombotic effects of tetramethylpyrazine in in vivo experiments. Int. J. Hematol. 73, 393–398.

Shih, Y.H., Wu, S.L., Chiou, W.F., Ku, H.H., Ko, T.L., Fu, Y.S., 2002. Protective effects of tetramethylpyrazine on kainate-induced excitotoxicity in hippocampal culture. Neuroreport 13, 515–519.

Tsai, T.H., 2003. Assaying protein unbound drugs using microdialysis techniques. J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 797, 161–173.

Tsai, T.H., Liang, C., 2001. Pharmacokinetics of tetramethylpyrazine in rat blood and brain using microdialysis. Int. J. Pharm. 216, 61–66.

Tuttle, R.S., Marmelstein, L., Trad, T., Reddy, S., Radley, T., 1989. In vitro uterine response to tetramethylpyrazine, the active constituent of chung chong (a traditional Chinese medicine). Am. J. Obstet. Gynecol. 161, 1319–1323.

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

Williams, F.G., Mullet, M.A., Beitz, A.J., 1995. Basal release of Met-enkephalin and neurotensin in the ventrolateral periaqueductal gray matter of the rat: a microdialysis study of antinociceptive circuits. Brain Res. 690, 207–216.
Xue, Q.F., Dai, S.L., Yuan, S.Y., Zhu, L.X., Wu, Y.Q., Wang, S.S., Li, C.C., Liu, C.Y., Wang, Z.Y., 1989. Effect of chuanxiongqin (tetrame-thylpyrazine) on microcirculatory perfusion in hamsters and capillary permeability in rats. Proc. Chin. Acad. Med. Sci. Peking Union Med. Coll. 4, 224–228.
Zhang, C., Wang, S.Z., Zuo, P.P., Cui, X., Cai, J., 2004. Protective effect of tetramethylpyrazine on learning and memory function in D-galactose-lesioned mice. Chin. Med. Sci. J. 19, 180–184.