BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **34**: 417–422 (2013)
Published online 14 August 2013 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/bdd.1854

Brain microdialysate, CSF and plasma pharmacokinetics of ligustrazine hydrochloride in rats after intranasal and intravenous administration

Qiao Wang*, Zhan Tang, and Wanggang Zhang
Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang, 310013, PR China

ABSTRACT: The aim of this work was to investigate the pharmacokinetics of ligustrazine hydrochloride (LZH) in plasma, cerebrospinal fluid (CSF) and cerebral cortex after intranasal (10 mg/kg) or intravenous administration (10 mg/kg) in male Sprague–Dawley rats. Plasma, CSF and cerebral cortex microdialysates were collected at timed intervals for the measurement of LZH by a quick and sensitive HPLC-UV method. LZH entered the brain quickly following both routes of administration. No significant difference was observed between the $AUC_{\text{CSF or cortex}}/AUC_{\text{plasma}}$ ratio of LZH after intranasal administration (38.4%, 17.4%) and that after intravenous injection (45.9%, 19.9%). The drug targeting index (DTI) was 0.85 and 0.91 in the CSF and cortex, respectively. In conclusion, LZH is rapidly absorbed into the systemic circulation following intranasal administration. There is no direct pathway for LZH transport from the nasal cavity to the brain. The rapidity and magnitude of LZH penetration into the brain indicate that intranasal administration of this agent is a promising alternative to intravenous administration. Copyright © 2013 John Wiley & Sons, Ltd.

Key words: ligustrazine hydrochloride; pharmacokinetics; intranasal administration; microdialysis; cerebrospinal fluid; plasma

Introduction

Ligustrazine, the active ingredient of the traditional herbal medicine *Ligusticum chuanxiong* Hort. or *Ligusticum wallichii* Franch, has significant therapeutic activity and can improve brain microcirculation, inhibit thrombus formation, decrease platelet aggregation and improve blood viscosity [1–3]. It has been used extensively in China as a drug for the treatment of ischemic cerebrovascular disease [1,4]. The clinically common form of application is ligustrazine hydrochloride (LZH) or ligustrazine phosphate and current market formulations are solutions for parenteral injection and tablets for oral delivery. But the oral bioavailability of ligustrazine is only about 30% [5].

Previous studies that measured the brain levels of ligustrazine in rats analysed brain homogenates [6,7], an approach that involves animal euthanasia at each sampling point. This requires a large number of animals and introduces a potential source of variation. Several investigations [8–11] used microdialysis probes implanted into the striatum. In the present study drug concentrations in brain microdialysis samples from the cortex and cerebrospinal fluid (CSF) were determined, because it is suggested that there may be differences in the mechanisms and rates of drug transport across the blood–brain and blood–CSF barriers. The pharmacokinetics of ligustrazine or ligustrazine phosphate in blood has also been studied using

* Correspondence to: Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang, 310013, PR China.
E-mail: wangqiao-1@163.com

Received 27 December 2012
Revised 5 July 2013
Accepted 11 July 2013

microdialysis [8,12]. However, the limited recovery achieved using microdialysis is a potential disadvantage. The plasma protein binding percentage of ligustrazine is about 70% [13]. Only unbound drug can permeate the microdialysis membrane and the concentrations may be low. Therefore drug levels were measured in plasma at the same time.

Intranasal delivery is a viable, non-invasive strategy for delivering drugs into the brain and, by avoiding first-pass metabolism, allows rapid onset of the therapeutic effect that can be important for centrally acting drugs used in crisis treatments. The present study aimed to investigate the pharmacokinetic characters of LZH in the three sites after intranasal and intravenous administration in rats. This approach allowed us to explore whether the nasal route can be used to transport LZH directly from the nasal cavity to the brain, and study the drug level in different regions (CSF, cortex and plasma).

Materials and Methods

Chemicals and reagents

Ligustrazine (LZH) (> 99.0%) was supplied by Shanghai Huamei Auxiliaries Factory (Shanghai, China). It was dissolved in water at a concentration of 150 mg/ml for nasal use and was diluted 20 times for intravenous use. High-performance liquid chromatography (HPLC) grade acetonitrile was obtained from Merck (Darmstadt, Germany). All other reagents were of commercially available analytical grades.

Animal experiments

Male Sprague–Dawley rats (250–300 g) were obtained from the Zhejiang Laboratory Animal Center (Hangzhou, China). Rats were anesthetized with an intraperitoneal dose of 1% (w/v) sodium pentobarbital (45 mg/kg). An intracerebral guide probe (BAS/MD-2251, USA) was implanted in the frontal cortex (coordinates: AP 2.1, ML 2.0, DV 1.0) according to the Paxinos and Watson atlas [14]. After surgery, the rats were placed in isolation for 7 days to recover and were given free access to food and water with a 12 h/12 h light/dark cycle prior to experimentation.

For *in vivo* measurements, the animals were fasted for 12 h and anesthetized with intraperitoneal 20% (w/v) urethane (1 g/kg); the body temperature was maintained at 37 °C using a heating pad. Collection of CSF was performed using cistern puncture as described in our previous study [15]. For brain microdialysis, the rats were perfused with Ringer's solution (144 mM NaCl, 4 mM KCl, 1.3 mM $CaCl_2$; pH 7.2). Brain microdialysis systems comprised a MD-1020K microinjection pump (BAS, USA) and microdialysis probes (MD-2204, membrane length 4 mm; BAS, USA). Recovery of the probe (R_{dial}) was carried out using an *in vitro* method as described previously [16]. After washing with Ringer's solution at a flow rate of 2.5 μl/min, the microdialysis probe was inserted into the frontal cortex through the guide probe; the system was allowed to equilibrate for 1 h before drug administration.

Approximately 20 μl of the nasal formulation (150 mg/ml LZH in solution) was administered via micropipette into the left nostril of the rat at a dose of 10 mg/kg. For intravenous administration, LZH solution at 7.5 mg/ml was applied at a dose of 10 mg/kg via the femoral vein in a volume of approximately 0.4 ml. The CSF and blood samples were collected at 2, 5, 10, 20, 40, 60, 90, 120, 150 and 180 min after dosing. Blood samples (0.1 ml) were taken from the tail vein and placed in a heparinized polyethylene (PE) conical tube and centrifuged at 10000 rpm for 10 min; in excess of 40 μl of plasma was obtained by this technique. Aliquots of CSF samples (25 μl) were collected using a microsyringe. For brain microdialysates the sampling interval was 10 min. All samples were placed in PE tubes and stored at −20 °C before analysis.

Aliquots of 60 μl of acetonitrile were added to 40 μl plasma samples to precipitate protein. Mixtures were vortexed for 2 min before centrifugation at 10000 rpm for 10 min. Supernatant was injected into the HPLC system using a 20 μl fixed loop. The CSF and brain microdialysate samples were analysed directly.

Analytical procedures and data analysis

The HPLC system comprised an LC-10A pump, a SPD-10A UV detector, an SCL-10A system controller (Shimadzu, Japan), a N2000 chromatographic workstation (Intelligent Information Engineer Ltd

DOI: 10.1002/bdd

of Zhejiang University) and a Diamonsil C18 column (4.6 mm × 150 mm, 5 μm, Dikma). A mixture of acetonitrile:water (35:65 v/v) was employed as the mobile phase with a flow rate of 1.0 ml/min. The wavelength of the UV detector was set at 281 nm and the temperature of the column oven was maintained at 35 °C.

The results obtained from the HPLC analyses were separately plotted as concentration versus time curves for plasma, CSF and cortical microdialysates. Concentrations of LZH in the brain (C_r) were calculated from the concentrations in the microdialysates (C_m) using the following equation: $C_r = C_m / R_{dial}$. Calculations of the pharmacokinetic parameters were performed on each individual set of data using DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China).

To evaluate the brain-targeting after nasal dosing, the drug targeting index (DTI) [17,18] was described as the ratio of the value of $AUC_{\text{CSF or cortex}}/AUC_{\text{plasma}}$ following i.n. administration to that following i.v. injection. The higher the DTI, the higher the degree of LZH targeting to the brain that can be expected after i.n. administration.

$$\text{DTI} = \frac{\left(\text{AUC}_{\text{CSF or cortex}}/\text{AUC}_{\text{plasma}}\right)_{\text{i.n.}}}{\left(\text{AUC}_{\text{CSF or cortex}}/\text{AUC}_{\text{plasma}}\right)_{\text{i.v.}}}$$

Results and Discussion

Concentration–time profiles in rat plasma, CSF and cortex after intranasal or intravenous administration of LZH are shown in Figure 1; the calculated pharmacokinetic parameters of LZH in rat plasma, CSF and cortex are presented in Table 1. *In vitro* probe recovery experiments showed no differences between the individual probes used in the study. The mean value of R_{dial} for LZH for all the microdialysis probes was 0.333 ± 0.032 at a microdialysate flow rate of 2.5 μl/min at 37 °C.

Intravenous injection of LZH generated plasma, CSF and cortex concentration–time profiles that were similar to those obtained following intranasal administration. The $AUC_{\text{CSF or cortex}}/AUC_{\text{plasma}}$ ratios after intranasal delivery (38.4%, 17.4%) did not differ significantly from the ratios observed after intravenous injection (45.9%, 19.9%) (Table 1). This indicated that, by both routes of administration, the CSF concentrations of LZH achieved approximately 40% of the levels seen in plasma, while cortical levels were approximately 20% of plasma levels.

One possible drawback of microdialysis sampling for the evaluation of systemic pharmacokinetics is that the first analyte concentration was obtained at the midpoint of the first collection interval [19,20]. When the intravenous bolus pharmacokinetics of a substance with rapid distribution into peripheral tissues is studied, the first usable analyte concentration may only be obtained at 5 min (in the case of a 10 min collection interval) following administration, or possibly later. This may therefore not provide an accurate description of the early distribution of the substance. In the present study we therefore analysed serial plasma, CSF and cerebral cortex microdialysate in parallel.

Several previous reports have addressed the brain microdialysis of ligustrazine or LZH or ligustrazine phosphate. Some reported on the concentrations of LZH or ligustrazine phosphate in the striatum, but reported no data in blood or CSF [9–11]. Others reported on the concentrations of ligustrazine or ligustrazine phosphate, in striatal and blood microdialysates [8,12]. Li *et al*. [9,10] studied LZH only in rat brain microdialysate after i.v. and i.n. administration (10 mg/kg). The $AUCs_{0-\infty}$ in brain of the two routes were very close to our results. But other pharmacokinetic parameters showed some differences. The reason may be that a different brain region was studied and the rats were awake during the experiment. In contrast to our results, these reports [8–12] showed that the second point analyte concentration was highest in striatal microdialysates, while in our study the first point concentration was the highest (Figure 1C). The reason for this discrepancy is unknown, but may reflect microdialysis of different brain regions. Two prior blood microdialysis studies also reported that blood concentrations were lower than those in the brain, because only unbound drug can permeate the microdialysis membrane, these reports suggested that the concentration of free drug in blood was low.

The CSF concentrations of LZH were highest at the second time point, and T_{max} was approximately

DOI: 10.1002/bdd

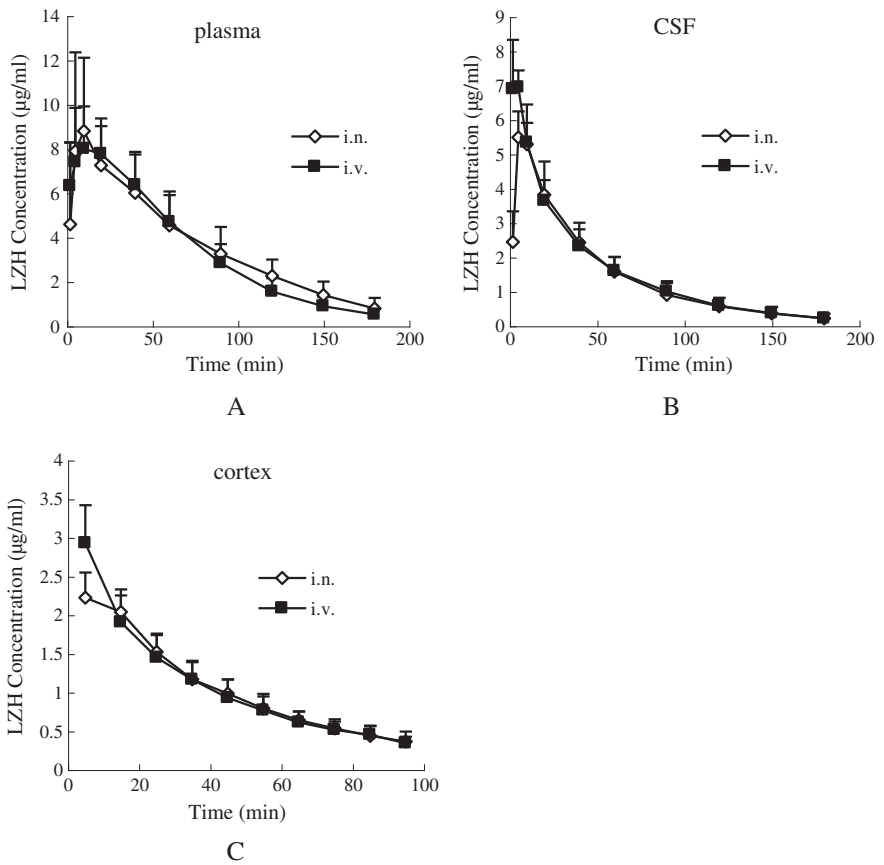

Figure 1. (A) Concentration versus time profiles of ligustrazine (LZH) in rat plasma after intravenous and intranasal administration ($n = 6$). There were no significant concentration differences at any time point between the two routes of administration. (B) Concentration versus time profiles of LZH in rat CSF after intravenous and intranasal administration ($n = 6$). Only the concentrations at the first two time points were significantly different ($p < 0.05$). (C) Concentration versus time profiles of LZH in rat cortex after intravenous and intranasal administration ($n = 6$). Only the concentrations at the first time point were significantly different ($p < 0.05$)

5 min, irrespective of the route of administration (Figure 1B), indicating that the drug is rapidly transported into the brain. The highest drug concentrations in the brain microdialysis samples were recorded at the first time point. This also indicates rapid transport of the drug into the brain, even though microdialysis samples were obtained over a specified time interval and reflect the 'average' concentrations of analyte during the collection interval (10 min).

The absorption of LZH from the nasal cavity into the systemic circulation was rapid and complete (Figure 1A). We report here that, unlike the classic drug concentration profile following intravenous administration, the highest point concentration of LZH in the plasma concentration–time profile after intravenous administration was at the third sampling point (10 min). This may reflect the unusual pharmacokinetic properties of the specific molecule measured, or possibly the blood sampling method which was withdrawn from the tail vein. In our prior studies with gastrodin [15], huperzine A [21], *p*-hydroxybenzyl alcohol [22] and other investigations with meptazinol [23], drug concentrations at the first time point were the highest after intravenous administration. Blood samples in these studies were also obtained via the tail vein and did not show unusual pharmacokinetic phenomena. Maybe LZH distribution to the tail vein was delayed after femoral vein administration. In another

DOI: 10.1002/bdd

Table 1. Non-compartmental pharmacokinetic parameters for LZH in rat plasma, CSF and cortical microdialysate after intranasal and intravenous administration (10 mg/kg, mean ± SD, $n = 6$)

Parameter	Intranasal (i.n.)			Intravenous (i.v.)		
	Plasma	CSF	Cortex	Plasma	CSF	Cortex
$AUC_{0\text{-}\infty}$ (min μg/ml)	742.1 ± 157.1	284.6 ± 70.5	129.0 ± 31.8	661.7 ± 103.1	301.6 ± 53.9	127.1 ± 21.8
$t_{1/2}$ (min)	49.2 ± 20.5	40.3 ± 9.2	39.0 ± 20.6	37.7 ± 8.3	40.4 ± 7.2	34.9 ± 5.9
C_{max} (μg/ml)	9.4 ± 4.1	5.9 ± 0.7[a]	2.3 ± 0.3[a]	8.3 ± 2.1	7.4 ± 1.0	2.9 ± 0.5
T_{max} (min)	9.2 ± 2.0	6.7 ± 2.6	8.3 ± 5.1	14.2 ± 6.6	4.0 ± 1.5	5.0 ± 0.0
$MRT_{0\text{-}\infty}$ (min)	76.8 ± 28.4	54.7 ± 12.0	53.8 ± 24.3	61.7 ± 13.8	52.6 ± 10.6	45.9 ± 5.0
CL_z/F (l/min/kg)	0.014 ± 0.003	-	-	0.016 ± 0.002	-	-
V_z/F (l/kg)	0.989 ± 0.484	-	-	0.841 ± 0.224	-	-
AUC_{cortex}/AUC_{CSF} (%)	-	-	46.3 ± 9.2	-	-	43.4 ± 11.7
$AUC_{CSF\ or\ cortex}/AUC_{plasma}$ (%)	-	38.4 ± 6.2	17.4 ± 2.7	-	45.9 ± 7.0	19.9 ± 6.0
DTI	-	0.85 ± 0.09	0.91 ± 0.17	-		

[a] $P < 0.05$ i.n. versus i.v.

study [6] the plasma concentration of intravenous ligustrazine was reported to achieve a peak at the first time point followed by an exponential decline in blood samples withdrawn via heart puncture, which was different to our study. Therefore, it is possible that blood samples withdrawn directly via heart puncture in rats could avoid this problem (or phenomenon). But this method was not used in our study because then brain microdialysiate, CSF and blood could not be sampled simultaneously.

In conclusion, LZH was rapidly and totally absorbed into the systemic circulation following intranasal administration. The DTIs were near 1. Because there were no significant differences between the two routes of administration, intranasal administration is a promising alternative route for the administration of LZH for brain therapy. The method is also non-invasive, permits the rapid onset of therapeutic effect and avoids first-pass hepatic metabolism.

Acknowledgements

The project was supported by grants from Zhejiang Provincial Natural Science Foundation (LY12H29011), Zhejiang Provincial Science and Technology Foundation (2011F10048), the Cultivation of High-level Innovative Health talents (XKQ010012008) and Medical Key Subject of Zhejiang Province (XKQ-010-001).

Conflict of Interest

The authors have declared that there is no conflict of interest.

References

1. Wu GF, Wang JP. Advances on the pharmacological effects and clinical applications of ligustrazine. *Chin Remed Clin* 2006; **6**: 773–774.
2. Na X, Wang XL, Pi RB. Advances in pharmacological research of ligustrazine on central nervous system. *Trad Chin Drug Res Clin Pharm* 2008; **19**: 77–80.
3. Ren ZQ, Sun LJ. Cardiovascular pharmacological effects of ligustrazine. *Chin J Integr Med Cardio-/Cerebrovasc Dis* 2008; **6**: 570–572.
4. Xu H, Shi DZ, Guan CY, Chen ZT. Clinical application and pharmacological actions of ligustrazine. *Chin J Integr Trad Western Med* 2003; **23**: 376–379.
5. Lou YQ, Zhang H, Cao X, Chen ML. The pharmacokinetics and disposition of tetramethylpyrazine phosphate in dogs and rats. *Acta Pharm Sin* 1986; **21**: 481–487.
6. Liang CC, Hong CY, Chen CF, Tsai TH. Measurement and pharmacokinetic study of tetramethylpyrazine in rat blood and its regional brain tissue by high-performance liquid chromatography. *J Chromatogr B* 1999; **724**: 303–309.
7. Liu YD, Sun HJ, Li R, Wu W, Chen HG. Influence of borneol on nasal absorption of ligustrazine. *China J Chin Materia Medica* 2008; **33**: 259–261.
8. Tsai TH, Liang CC. Pharmacokinetics of tetramethylpyrazine in rat blood and brain using microdialysis. *Int J Pharm* 2001; **216**: 61–66.
9. Li FZ, Feng YR, Feng J. Studies on brain pharmacokinetics of tetramethylpyrazine hydrochloride after intranasal administration in rats. *Chin Pharm J* 2008; **43**: 452–454.

10. Feng J, Li F, Zhao Y, Feng Y, Abe Y. Brain pharmacokinetics of tetramethylpyrazine after intranasal and intravenous administration in awake rats. *Int J Pharm* 2009; **375**: 55–60.
11. Yan YL, Ye Y, Zhou LL. Study on brain pharmacokinetics of tetramethylpyrazine phosphate after intranasal administration. *Trad Chin Drug Res Clin Pharm* 2009; **20**: 548–550.
12. Yan YL, Liu HW, Zhou LL. Pharmacokinetics of nasal tetramethylpyrazine phosphate pH-sensitive *in situ* gel in rat blood and brain. *Chin Pharm J* 2012; **47**: 119–122.
13. Ye Y, Zhou LL, Yan YL, Huang QJ. Determination of plasma protein binding rate of tetramethylpyrazine phosphate by ultrafiltration. *J Chin Med Mater* 2010; **33**: 1282–1285.
14. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates (4th edn). Academic Press, Inc.: San Diego, CA, 1998; 118–132.
15. Wang Q, Chen G, Zeng S. Pharmacokinetics of gastrodin in rat plasma and CSF after i.n. and i.v. *Int J Pharm* 2007; **341**: 20–25.
16. Wang Q, Chen G, Zeng S. Distribution and metabolism of gastrodin in rat brain. *J Pharm Biomed Anal* 2008; **46**: 399–404.
17. Wang F, Jiang X, Lu W. Profiles of methotrexate in blood and CSF following intranasal and intravenous administration to rats. *Int J Pharm* 2003; **263**: 1–7.
18. Wang D, Gao Y, Yun L. Study on brain targeting of raltitrexed following intranasal administration in rats. *Cancer Chemother Pharmacol* 2006; **57**: 97–104.
19. Stahle L. Microdialysis in pharmacokinetics. *Eur J Drug Metab Pharmacokinet* 1993; **18**: 89–96.
20. Verbeeck RK. Blood microdialysis in pharmacokinetic and drug metabolism studies. *Adv Drug Deliver Rev* 2000; **45**: 217–228.
21. Wang Q, Chen GS. Pharmacokinetic behavior of huperzine A in plasma and cerebrospinal fluid after intranasal administration in rats. *Biopharm Drug Dispos* 2009; **30**: 551–555.
22. Wang Q, Chen GS. Pharmacokinetics of gastrodigenin in rat plasma and CSF after intranasal and intravenous administration. *Chin J New Drugs* 2010; **19**: 708–712.
23. Shi Z, Zhang Q, Jiang X. Pharmacokinetic behavior in plasma, cerebrospinal fluid and cerebral cortex after intranasal administration of hydrochloride meptazinol. *Life Sci* 2005; **77**: 2574–2583.

DOI: 10.1002/bdd