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Short communication

Distribution and metabolism of gastrodin in rat brain

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Abstract

Gastrodin is the major and bioactive component in Tianma (*Gastrodia elata* Bl.) and has sedative, anticonvulsive and neuroprotective effects. Since little is known about its neuropharmacokinetics and brain metabolism, this study was undertaken to investigate the kinetic inter-relationship of gastrodin in rat plasma, cerebrospinal fluid (CSF) and brain microdialysate (frontal cortex, hippocampus, thalamus and cerebellum). Gastrodin was administered via the femoral vein at a dose of 200 mg/kg, and blood, CSF and brain microdialysate were collected at timed intervals for the measurement of gastrodin concentrations by high-performance liquid chromatography. The samples were analyzed on a Diamonsil C18 column (5 μm, 250 mm × 4.6 mm i.d.) with a mobile phase consisting of acetonitrile–water (5% acetonitrile for brain microdialysate, 2.5% acetonitrile for plasma and CSF), and detected with a UV detector at 221 nm. The distribution of gastrodin in rat showed that levels of gastrodin declined rapidly after drug administration, and the entry of gastrodin into the brain was rapid. However, the ratios of AUC_{brain}/AUC_{plasma} were not high. The individual ratios of the AUC in the CSF, frontal cortex, hippocampus, thalamus and cerebellum to the AUC in the plasma were 4.8 ± 2.4%, 3.3 ± 1.2%, 3.0 ± 0.7%, 3.3 ± 1.3% and 6.1 ± 1.9%, respectively. The AUC in the cerebellum was significantly higher than that in other brain regions ($P < 0.05$). The concentrations of *p*-hydroxybenzyl alcohol, the main metabolite of gastrodin, were very low both in the CSF and plasma.
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Keywords: Gastrodin; *p*-Hydroxybenzyl alcohol; Microdialysis; Cerebrospinal fluid; Brain distribution; Metabolism

1. Introduction

Gastrodin (Gas, Fig. 1) is one of the major and bioactive components in Tianma (*Gastrodia elata* Bl.) and has shown sedative, anticonvulsive and neuroprotective effects [1–6]. It has been approved as a drug for the treatment of neurasthenia, dizziness, headache and as adjunctive therapy for epilepsy in China. The results of recent clinical trials showed that it is efficient in the treatment of patients with vascular dementia.

p-Hydroxybenzyl alcohol (HBA, Fig. 1) is the main metabolite of Gas, and is also one of the components in Tianma (*G. elata* Bl.) and has pharmacological effects similar to Gas [1,7,8].

There have been some reports about the quantitative methods for measuring Gas and its metabolite HBA in biological samples; these methods require the use of extraction steps, many organic solvents and an internal standard [9–13]. However, little is known about its neuropharmacokinetics and brain metabolism, and it is important to quantify Gas in rat brain.

To measure Gas in the brain, previous studies [14,15] reported using ^{3}H-Gas, which involved sacrificing the rats or mice at each sampling point, so that many animals were needed and the results may have been prone to more error. Microdialysis has economical and ethical advantages, making it a powerful sampling technique for the study of the local actions of drugs in different tissues, especially in brain regions. In a recent study [16], microdialysis was used to measure Gas in the brain striatum and blood. However, the limited recovery achieved using microdialysis may be a potential disadvantage. The plasma protein-binding ratio of Gas is 4.3%, while that of HBA is 69.3% [14]. Only unbound drug can permeate the microdialysis membrane, and the concentration of HBA may be too low to be detected; therefore the levels of Gas and HBA in the plasma were determined. The concentration of drug in the cerebrospinal fluid (CSF) can reflect that in the brain, so the concentrations of Gas and HBA in the CSF were also determined. In the present study, microdialysate and CSF were analyzed directly. Based on our previous study [17],

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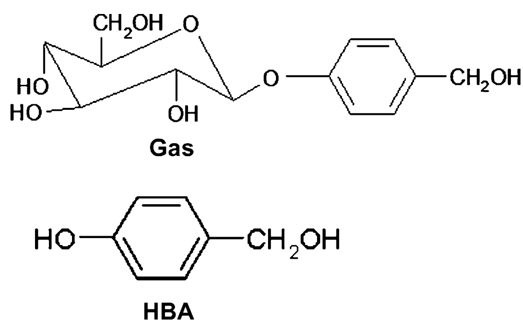

Fig. 1. Structure of Gas and HBA.

the plasma supernatant after precipitation of protein by perchloric acid can also be analyzed directly, thereby simplifying the pre-processing of biosamples.

The aim of this study was to establish a simple and sensitive method for the simultaneous determination of Gas and its metabolite HBA, by which to investigate the distribution and metabolism of Gas in the rat brain and to find out which brain region is most influenced by Gas.

2. Experimental

2.1. Chemicals and reagents

Gas (>99.0%) was supplied by Huizhou Orient Plant Health Care Sci.&Tech. Co. (Guangdong, China), and dissolved in water at a concentration of 100 mg/ml for intravenous use. HBA (>99.5%) was supplied by Yizheng Dixin Chemical Co., Ltd. (Jiangsu, China). Acetonitrile of high-performance liquid chromatography (HPLC) grade was obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade and commercially available.

2.2. Chromatography

The HPLC system consisted of an LC-10A pump, SPD-10A UV detector, SCL-10A system controller (Shimadzu, Japan), N2000 chromatographic workstation (Intelligent Information Engineer Ltd. of Zhejiang University), and a Diamonsil C18 column (4.6 mm × 250 mm, 5 μm, Dikma). A mixture of acetonitrile–water was employed as a mobile phase, with a flow-rate of 1.0 ml/min (5% acetonitrile for the microdialysate, 2.5% acetonitrile for the plasma and CSF). The wavelength of the UV detector was set at 221 nm and the temperature of the column oven was maintained at 33 °C.

2.3. Analytical method validation and sample preparation

A stock solution containing 2.287 mg/ml Gas was prepared in water. This solution was diluted with water to prepare the working solutions. Linearity was assessed by analyzing 12 standards in plasma (0.28–571.70 μg/ml), nine standards in CSF (0.16–40.02 μg/ml) and nine standards in brain microdialysate (0.07–17.86 μg/ml). Similarly, a stock solution containing 2.360 mg/ml HBA was prepared in methanol. It was diluted with water to prepare the working solutions. Linearity was assessed by analyzing five standards in plasma (0.15–2.36 μg/ml), five standards in CSF (0.07–1.18 μg/ml) and five standards in brain microdialysate (0.04–0.59 μg/ml).

The calibration curve was based on drug peak area and was analyzed by weighted linear regression using the DAS 2.0 (Drug and Statistics for Windows) Program (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The lower limit of quantification (LLOQ) was defined as the lowest drug concentration producing at least five times the response compared to the blank response and with acceptable precision (R.S.D. < 20%) and accuracy (80–120% of nominal concentration). Intra- and inter-day precision and accuracy were determined by analyzing spiked samples at three different concentrations on five different days. Each concentration experiment was conducted in six replicates from sample preparation to chromatographic analysis. Three aliquots at each of the low and high concentrations of the spiked samples were stored at the intended storage temperature and time to study the stability of Gas and HBA according to the FDA guidance [18].

Aliquots of 50 μl of 6% perchloric acid were added into the 50 μl blank, control or plasma sample to precipitate protein. The mixture was vortexed for 1 min and centrifuged at 9000 rpm for 10 min. The supernatant was immediately injected into the HPLC system with a 20 μl fixed loop. The CSF and brain microdialysate samples were analyzed directly.

2.4. Animal experiment

Male Sprague–Dawley rats (250–300 g) were obtained from the Zhejiang Laboratory Animal Center (Hangzhou, China). The rats were anesthetized with an intraperitoneal dose of 1% (w/v) sodium pentobarbital (45 mg/kg). Intracerebral guide probes (BAS/MD-2251, USA) were implanted in the frontal cortex (coordinates: AP 2.1, ML 2.0, DV 1.0) and hippocampus (AP −6.0, ML −4.6, DV 3.0), or thalamus (AP −3.0, ML 1.0, DV 4.5) and cerebellum (AP −11.0, ML −1.3, DV 2.0) according to the Paxinos and Watson atlas [19]. After surgery, the rat was placed in a single cage for 7 days for recovery. The positions of the probes were verified by a standard histological procedure at the end of the experiments.

On the day of the *in vivo* experiment, the rat was maintained anesthetized with an intraperitoneal dose of 20% (w/v) urethane (1 g/kg), and the body temperature was kept at 37 °C by a heating pad. Collection of CSF was performed using the cistern puncture, in accordance with our previous study [17]. For brain microdialysis, the rat was perfused with Ringer's solution (144 mM Na^+, 4 mM K^+, 1.3 mM Ca^+; pH 7.2), which was prepared in ultra pure deionized water and filtered through a 0.45-μm nylon filter before use. Brain microdialysis systems consisted of a MD-1020K microinjection pump (BAS, USA) and microdialysis probes (MD-2204, membrane length 4 mm, BAS, USA). Each probe was subjected to *in vitro* recovery studies before *in vivo* experiments for validation. After being washed with Ringer's solution at a flow-rate of 2.5 μl/min, two microdialysis probes were each inserted into one of two different brain

regions in the rat through the guide probe, and the whole system was allowed to equilibrate for 0.5 h before drug administration.

2.5. Drug administration and sample collection

The Gas solution at a dose equivalent to 200 mg/kg was administered via the femoral vein. At 2, 5, 10, 20, 30, 40, 60, 90, 120, 150,180, 210 and 240 min after dosing, CSF and blood samples were collected. A 0.15-ml blood sample was taken from the tail vein and placed in a heparinized polyethylene (PE) conical tube, then centrifuged at 9000 rpm for 10 min; by this technique, more than 50 μl of plasma was obtained. Aliquots of 25-μl CSF samples were collected by using a microsyringe. The CSF and plasma samples were placed in a PE tube, stored at –20 °C and analyzed as soon as possible.

For the brain microdialysis, the sampling interval was 10 min. The brain microdialysates were immediately measured by a validated HPLC system.

2.6. Recovery of the probe

Recovery of the probe (R_{dial}) was carried out using an *in vitro* method [20–22]. Each microdialysis probe was placed in a beaker containing a stirred standard solution of 5-μg/ml Gas or 2-μg/ml HBA thermostated at 37 °C and perfused with drugs free Ringer's solution at a flow-rate of 2.5 μl/min. Samples were collected every 20 min for 60 min. The concentrations of Gas or HBA in dialysate (C_{dial}) or in standard solution (C_{Stand}) were determined by HPLC. R_{dial} was calculated as $R_{dial} = C_{dial}/C_{Stand}$.

2.7. Gas distribution and metabolism

Gas microdialysate concentrations (C_m) in the brain were converted to real concentration (C_r) as follows: $C_r = C_m/R_{dial}$. The calculations of pharmacokinetic parameters were performed on each individual set of data using the software DAS 2.0 Program. Analysis of variance was used to study the statistical differences, and a value of $P < 0.05$ was consid-

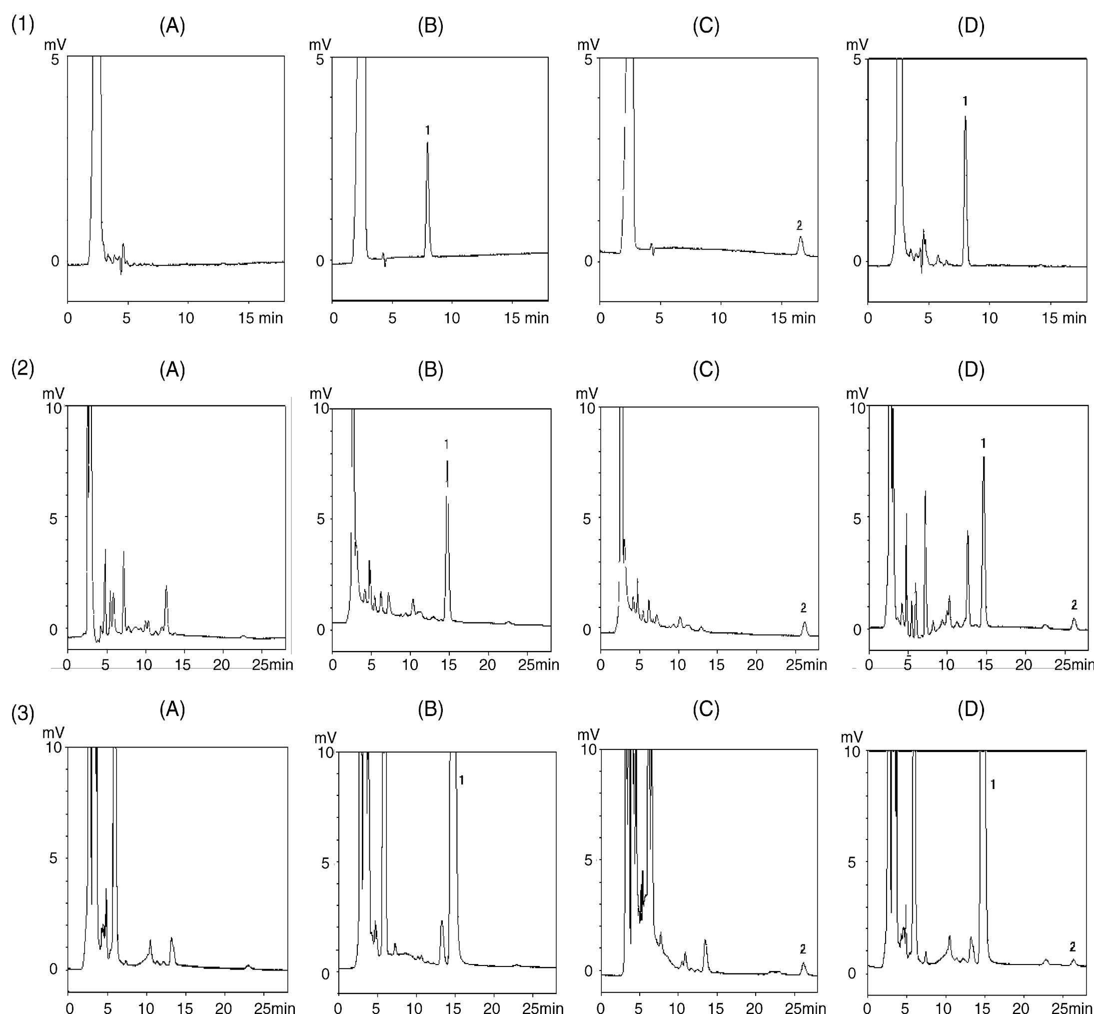

Fig. 2. (1) Chromatograms of (A) blank brain dialysate from the microdialysis probe before drug administration; (B) Ringer's solution spiked with 2.23 μg/ml Gas; (C) Ringer's solution spiked with 0.29 μg/ml HBA; (D) the brain dialysate sample collected at 30 min after Gas administration. 1 = Gas; 2 = HBA. (2) Chromatograms of (A) blank CSF; (B) CSF spiked with 10.0 μg/ml Gas; (C) CSF spiked with 0.59 μg/ml HBA; (D) the CSF sample collected at 30 min after Gas administration. 1 = Gas; 2 = HBA. (3) Chromatograms of (A) blank plasma; (B) plasma spiked with 285.8 μg/ml Gas; (C) plasma spiked with 1.18 μg/ml HBA; (D) the plasma sample collected at 10 min after Gas administration. 1 = Gas; 2 = HBA.

ered statistically significant (Computer Program SAS, version 6.12).

3. Results and discussion

3.1. Validation

3.1.1. Selectivity

It was shown that the maximum absorption wavelengths of Gas and HBA are at 221 nm, by checking their UV spectra. Typical chromatograms are shown in Fig. 2. No peaks interfered with the analytes in the chromatograms of plasma and brain samples. The retention time of Gas was about 14.6 min in the 2.5% acetonitrile mobile phase and 8 min in the 5% acetonitrile mobile phase. The retention time of HBA was about 26.3 min in the 2.5% acetonitrile mobile phase and 16.7 min in the 5% acetonitrile mobile phase. Fig. 2(1D), (2D) and (3D) shows the chromatogram after intravenous administration of Gas. No HBA could be detected in the microdialysate.

3.1.2. Linearity and sensitivity

The calibration curves of Gas were linear over the investigated concentration ranges in plasma, CSF and brain microdialysate ($n = 5$). The regression equations (weight = $1/X^2$) where $Y = 9405.669X + 1033.537$(plasma), $Y = 18680.441X + 384.061$(CSF), $Y = 19364.342X + 524.802$(brain microdialysate). The correlation coefficients were higher than 0.999, indicating good linearity. The LLOQs of Gas were 0.28 μg/ml in plasma, 0.16 μg/ml in CSF and 0.07 μg/ml in brain microdialysate ($n = 5$, R.S.D. < 12.4%).

The calibration curves for HBA were also linear over the investigated concentration ranges in plasma, CSF and brain microdialysate ($n = 3$). The correlation coefficients were higher than 0.999. The regression equations (weight = $1/X^2$) where $Y = 16588.890X + 401.401$(plasma), $Y = 37932.843X + 156.191$(CSF) and $Y = 37134.181X + 200.178$(brain microdialysate). The LLOQs of HBA were 0.15 μg/ml in plasma, 0.07 μg/ml in CSF and 0.04 μg/ml in brain microdialysate ($n = 5$, R.S.D. < 13.7%).

The calibrators samples were not prepared containing both Gas and HBA. The one reason is the difference of the stability of Gas and HBA. The results from our previous experiments indicated that some Gas will be decomposed to HBA when it is at the unsuitable condition, so we analyzed Gas or HBA in calibrators' samples separately. The other reason is that Gas is over a wide concentration range, while HBA is over very narrow concentration range.

3.1.3. Precision and accuracy

The intra- and inter-assay precision and accuracy of Gas and HBA were found to be acceptable for the analysis (Table 1). The data were based on assay of six replicates on five different days. The R.S.D.s of Gas and HBA in rat plasma, CSF and brain microdialysate at three different concentrations were all less than 12.4% and 13.7%, respectively. The accuracy of Gas and HBA in rat plasma, CSF and microdialysate were in the range 91.6–104.5% and 94.2–104.0%, respectively.

Table 1
Precision (R.S.D.) and accuracy for the determination of Gas or HBA in rat plasma, CSF and microdialysate (data were based on assay of six replicates on five different days)

	Concentration spiked (μg/ml)	R.S.D. (%)		Accuracy (%)
		Intra-day	Inter-day	
Gas				
Plasma	0.56	6.3	7.0	101.7
	17.87	2.7	4.4	96.8
	285.85	2.0	3.1	97.6
CSF	0.16	7.4	9.4	91.6
	2.57	3.7	5.0	101.3
	20.58	1.9	2.1	98.9
Microdialysate	0.07	10.8	12.4	102.5
	1.12	4.3	5.2	104.5
	8.93	3.2	3.9	104.1
HBA				
Plasma	0.15	10.5	13.7	94.2
	0.59	2.4	4.4	102.0
	2.36	1.4	2.0	99.9
CSF	0.07	9.1	10.5	97.0
	0.30	3.3	4.8	102.1
	1.18	2.8	2.9	96.2
Microdialysate	0.04	13.4	9.5	104.0
	0.15	7.4	7.4	102.7
	0.59	1.7	1.5	100.0

3.1.4. Stability

The stability of Gas in biosamples was investigated under different storage and process conditions. The analytes were assayed at low and high concentrations (2.23 and 285.85 μg/ml for plasma, 0.63 and 20.01 μg/ml for CSF, and 0.28 and 8.93 μg/ml for microdialysate) in triplicate. The analytes were found to be stable after 10 h at room temperature (93.0–104.0% of the initial value) or three freeze–thaw (−20 °C/room temperature) cycles in 2 weeks (91.7–105.6% of the initial value). No HBA was detected.

The stability of HBA in biosamples was also investigated under the conditions described above. HBA was stable in brain microdialysate, CSF and plasma. However, HBA in plasma (0.15 and 2.36 μg/ml) was stable only for 2 h at room temperature (20 °C, 85.1–99.1% of the initial value) and for 6 h at 4 °C (86.2–97.4% of the initial value) when perchloric acid was added to precipitate protein in plasma samples. It was better to analyze the samples immediately after the precipitation of protein.

3.2. Pharmacokinetic study

The concentration versus time curves of Gas in rat plasma, CSF and brain microdialysate are shown in Fig. 3. The pharmacokinetic parameters of Gas in rat plasma, CSF and brain are presented in Table 2.

The *in vitro* probe recovery experiments showed no difference between the individual probes used in the study. The mean value of R_{dial} for Gas for all of the microdialysis probes was 0.209 ± 0.018 at a microdialysate flow-rate of 2.5 μl/min at

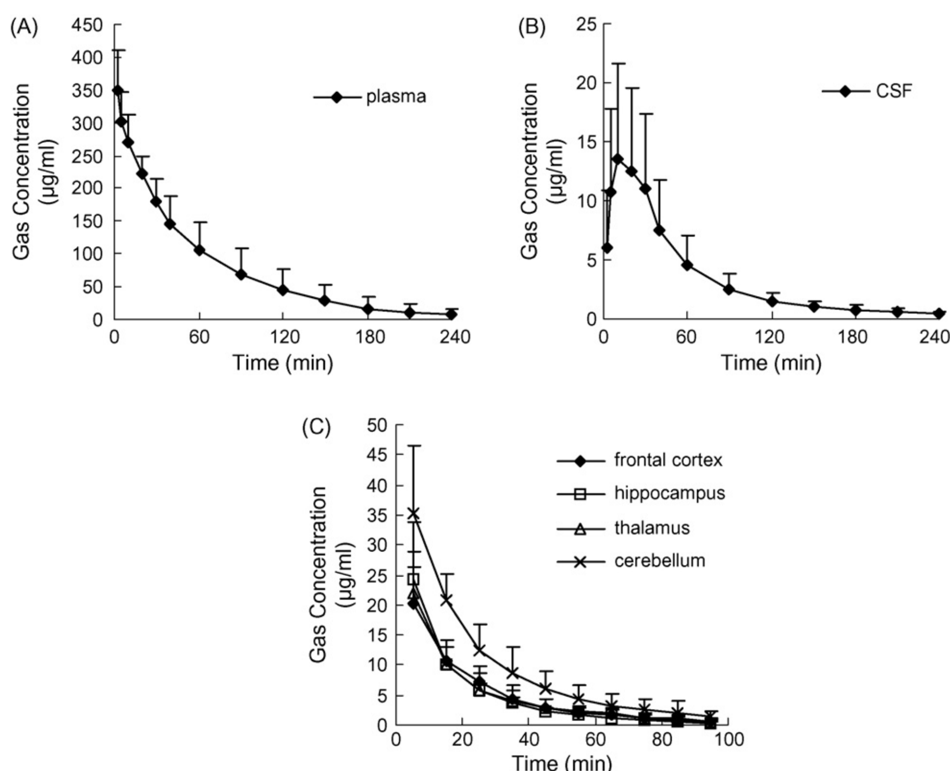

Fig. 3. (A) The concentration vs. time profile of Gas in rat plasma after i.v. administration ($n = 12$). (B) The concentration vs. time profile of Gas in rat CSF after i.v. administration ($n = 12$). (C) The concentration vs. time profiles of Gas in the rat brain after i.v. administration ($n = 6$). The concentration values at the first six time points in the cerebellum were higher than those of the corresponding time points in the other regions ($P < 0.05$).

37 °C. The *in vitro* R_{dial} for HBA was also about 0.2, but the concentration of brain microdialysate was lower than the LLOQ of HBA and could not be detected.

Some studies have determined the probe recovery using an *in vitro* method [20–22], and one report showed that the *in vivo* recovery of some compounds was in good agreement with the *in vitro* recovery [23]. The *in vivo* recovery was previously measured in our study, and no difference was observed between the *in vivo* and *in vitro* recovery of the probe for Gas. Therefore the simple *in vitro* recovery method was used in the present study.

The results indicated that the levels of Gas in plasma, CSF and brain declined rapidly after administration, and that Gas rapidly entered the brain tissues. The area under the curve (AUC) in the cerebellum was higher than that in other regions ($P < 0.05$), which suggested that Gas may have a more potent effect on the cerebellum. Whether or not the parameter of disposition is related to the pharmacological effect of the drug in the brain needs further study.

After i.v. administration of Gas, HBA formed immediately. However, the concentration was low and declined very quickly. The concentration of HBA was lower than the LLOQ after 60 min in plasma and 90 min in CSF, respectively.

Fig. 4 shows that the concentration of HBA in the CSF was higher than that in the plasma at 10, 20 and 30 min, but only

Table 2
The non-compartmental pharmacokinetic parameters for Gas in rat plasma, CSF and brain following i.v. administration (200 mg/kg)

Parameters	Mean ± S.D.					
	Plasma ($n = 12$)	CSF ($n = 12$)	Brain ($n = 6$)			
			Frontal cortex	Hippocampus	Thalamus	Cerebellum
$AUC_{0-\infty}$ (min μg/ml)	19019.7 ± 6801.2	848.9 ± 400.6	587.7 ± 185.0	549.7 ± 157.4	570.0 ± 188.3	1041.5 ± 259.2[a]
$t_{1/2}$ (min)	41.2 ± 15.6	64.7 ± 8.6	29.6 ± 19.0	25.6 ± 11.3	27.8 ± 14.6	25.2 ± 2.8
C_{max} (μg/ml)	350.9 ± 56.1	16.1 ± 7.7	21.6 ± 6.0	24.3 ± 9.4	22.0 ± 6.9	35.8 ± 10.3[a]
T_{max} (min)	–	15.0 ± 8.8	–	–	–	–
$MRT_{0-\infty}$ (min)	57.5 ± 23.4	66.4 ± 15.0	26.0 ± 8.4	22.7 ± 6.5	27.9 ± 11.8	26.8 ± 8.8
CLz/F (l/(min/kg))	0.012 ± 0.004	–	–	–	–	–
Vz/F (l/kg)	0.65 ± 0.20	–	–	–	–	–
AUC_{Brain}/AUC_{CSF} (%)	–	–	70.9 ± 29.1	66.4 ± 23.6	80.5 ± 30.1	155.0 ± 70.9[a]
$AUC_{CSF\ or\ Brain}/AUC_{plasma}$ (%)	–	4.8 ± 2.4	3.3 ± 1.2	3.0 ± 0.7	3.3 ± 1.3	6.1 ± 1.9[a]

[a] $P < 0.05$ vs. frontal cortex, hippocampus, thalamus.

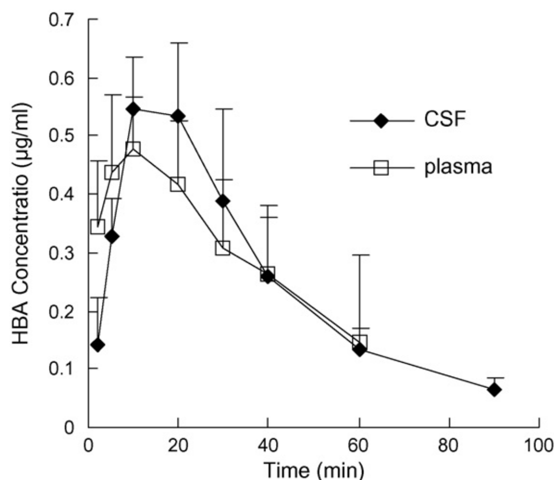

Fig. 4. The concentration vs. time profiles of HBA in rat CSF and plasma after Gas administration (i.v. 200 mg/kg) ($n = 12$).

the value at 20 min was statistically significant ($P < 0.05$). The $AUCs_{(0-t)}$ of HBA in the CSF and plasma were all very small, at 21.7 ± 5.6 and 18.2 ± 7.0 min μg/ml, respectively, without significant difference. The C_{max} and $t_{1/2}$ of HBA in CSF was 0.59 ± 0.11 μg/ml and 21.2 ± 2.8 min, respectively. It has been reported [14,15] that Gas is metabolized to HBA, and that the HBA then passes through the blood–brain barrier and gives rise to a pharmacological effect. However, the present study showed that Gas is able to pass through blood–brain barrier, and probably gives rise to its main pharmacological effect in the brain.

4. Conclusion

In the present study, a specific, sensitive and rapid HPLC method was developed for the simultaneous determination of Gas and its metabolite HBA in rat plasma, CSF and brain microdialysate. This method was then used to investigate the distribution and metabolism of Gas and HBA. The obtained data suggested that Gas rapidly enters the central nervous system, and the concentration of Gas in the cerebellum was found to be higher than that in other regions. The concentrations of HBA in the CSF and plasma were all very low and declined rapidly.

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