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Pharmacokinetics of tetramethylpyrazine in rat blood and brain using microdialysis

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Abstract

Since the central nervous acting agent, tetramethylpyrazine, is reported to have appreciable blood–brain barrier penetrability, a design allowing simultaneous and continual monitoring of drug concentrations in blood and brain was employed to study the distribution of intravenously administered tetramethylpyrazine (10 mg kg^{-1}). The system consisted of two microdialysis probes, each optimally constructed for sampling of the respective body fluids, inserted into the right jugular vein and striatum of male Sprague–Dawley rats. The probes were perfused with appropriate media at rates optimized for recovery. Dialysates were automatically collected using a microfraction collector and drugs were analyzed by high performance liquid chromatography (HPLC) with ultra violet (UV) detection. Results indicate that both blood and brain pharmacokinetics of unbound tetramethylpyrazine fit best to a two-compartment model. The elimination half-life of tetramethylpyrazine in rat blood and brain were 82.1 and 184.6 min, respectively. Increasing brain/blood concentration ratios suggested that tetramethylpyrazine effectively penetrated the blood–brain barrier.

Keywords: Tetramethylpyrazine; Microdialysis; Jugular vein; Striatum; Blood–brain barrier

1. Introduction

Tetramethylpyrazine is a biologically active ingredient isolated in 1957 from the traditional herbal medicine *Ligusticum wallichii* Franch. or *Ligusticum chuanxiong* Hort, which is widely used in China for the treatment of cardiovascular problems (Guo et al., 1983). It is believed to increase cerebral blood flow (Ojewole and Odebiyi, 1980; Feng et al., 1988) during ischemic attack (Ho et al., 1989). It has also recently been reported that tetramethylpyrazine may ameliorate learning deficits induced by permanent occlusion of the bilateral common carotid arteries (Ni, 1995) and effectively enhance cognition in rats (Watanabe, 1997). In addition, tetramethylpyrazine has been reported to have effects including improvement of cerebral blood

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flow (Ojewole and Odebiyi, 1980; Feng et al., 1988) during ischemic attack (Ho et al., 1989). These reports of central nervous system effects strongly indicate that tetramethylpyrazine may effectively penetrate the blood–brain barrier. Thus the present study examined the distribution of tetramethylpyrazine by continually monitoring concentrations of the unbound drug in the rat blood and brain using microdialysis, a method that causes minimal perturbance to the hemodynamics and physiological processes. The data thus obtained provides the basis for the construction of pharmacokinetic profiles and analyses.

Microdialysis is not without limitations, the chief of which is related to surgical trauma and recovery. Although several reports concluded that, by several hours after surgery, the blood–brain barrier is not affected around the microdialysis probe (Benveniste et al., 1987; de Lange et al., 1997), others found evidence for a reduced efficacy of this barrier (Major et al., 1990; Westergren et al., 1995). Goldsmith et al. (1995) indicate that 2 h after implantation of the microdialysis probe the BBB had been essentially re-established. Therefore, under optimum conditions, this technique allows continual in vivo sampling of free drug concentrations in plasma and extracellular space of most tissues without affecting the fluid volume and therefore the distribution dynamics (Hurd et al., 1988; Sato et al., 1996; Wright et al., 1996). In the present study, we coupled optimized microdialytic sampling with an effective liquid chromatographic assay to investigate the disposition of unbound tetramethylpyrazine in rat blood and brain following its intravenous administration (10 mg kg^{-1}).

2. Experimental

2.1. Chemicals

Tetramethylpyrazine (Fig. 1) was purchased from Aldrich Chemical Co. (USA). Methanol and chromatographic reagents were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore Corp., Bedford, MA) was used for all preparations. A standard stock solution of tetramethylpyrazine was prepared by dissolving 1.0 mg of tetramethylpyrazine in 10 ml of methanol.

2.2. Animals

Specific pathogen-free male Sprague–Dawley rats (300 ± 50 g) were obtained from the Laboratory Animal Center of The National Yang-Ming University, Taipei, Taiwan. For at least 1 week before use, they were kept in their own environmentally controlled quarters with temperature maintained at 24 ± 1°C and a light–dark cycle with light period from 7:00 to 19:00). Water and standard laboratory food were given ad libitum until 18 h before the experiments, at which time only food was withdrawn.

2.3. Microdialysis experiments

2.3.1. Microdialytic efficiency assessment

The microdialysis system consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden), appropriate dialysis probes through which perfusion fluid was pumped and a CMA-140 fraction collector for the collection of perfusates. Dialysis probes for blood and brain sampling were made of silica glass, concentric in design with 10

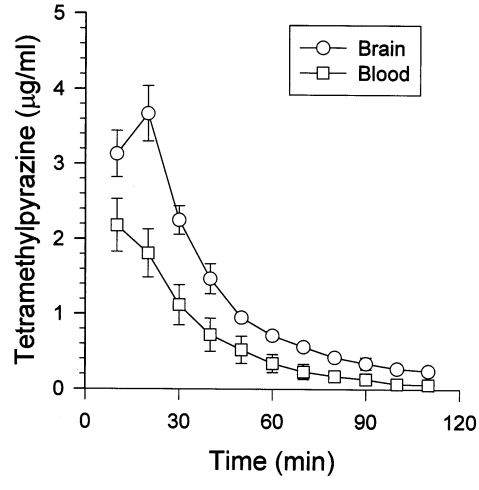

Fig. 1. Blood and brain concentrations vs. time profile of unbound tetramethylpyrazine after drug administration (10 mg kg^{-1}, i.v., $n = 4$).

and 3 mm dialyzing surfaces (150 μm diameter, nominal molecular weight cut-off 13 000 Da, Spectrum, CA, USA), respectively.

For in vivo recovery, microdialysis probes were inserted into either the jugular vein or striatum of sodium pentobarbital anesthetized animals (Hsiao et al., 1990; Bouw and Hammarlund-Udenaes, 1998). Ringer's (147 mM Na^+; 2.2 mM Ca^{2+}; 4 mM K^+; pH 7.0) or the anticoagulant ACD solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM for blood dialysis) containing tetramethylpyrazine (1 or 5 μg ml^{-1}) was pumped through the microdialysis probe at a constant flow rate (2 μl min^{-1}) using the microinjection pump (CMA/100). Following stabilization for 2 h after probe implantation, dialysates were collected for 1 h, and the inlet (C_{in}) and outlet (C_{out}) concentrations of tetramethylpyrazine determined by high performance liquid chromatography (HPLC). The in vivo recovery ratio (R_{vivo}) of tetramethylpyrazine across a microdialysis probe in the jugular vein or striatum was calculated by the following equation (Hsiao et al., 1990; Sato et al., 1996): $R_{vivo} = 1 - (C_{out}/C_{in})$.

2.4. Microdialysis experiments

Under sodium pentobarbital anesthesia, the rat's femoral vein was cannulated in preparation for intravenous drug administration. Following the insertion of a blood microdialysis probe into the right jugular vein, the rat was mounted on a Kopf stereotaxic frame for the implantation of a brain dialysis probe into the striatum using the co-ordinates (AP 0.2 mm; ML −3.2 mm; DV −7.0 mm) from the Paxinos and Watson (1982). Using the microinjection pump (CMA/100), the blood and brain dialysis probes were perfused with the anticoagulant ACD and Ringer's solutions, respectively, at flow-rates of 2 μl min^{-1} (Tsai and Chen, 1994, 1996). Following a post-surgical stabilization of the dialysate levels (approximately 2 h, Goldsmith et al., 1995), drug-free samples were collected into the microfraction collector and tetramethylpyrazine (10 mg kg^{-1}) was then intravenously administered via the femoral vein. For pharmacokinetic studies, dialysis samples were collected every 10 min and 20 μl for an additional 2 h and the dialysates assayed by HPLC. Body temperatures of the animals were maintained at 37°C throughout the experiment by using a heating pad. The position of the striatal probe was verified by standard histological procedures at the end of the experiment (Tsai and Chen, 1994).

2.5. Drug determination and pharmacokinetic analyses

Drug levels were determined by HPLC, which consisted of a Rheodyne injector, a chromatographic pump (ICI 1100, ICI Instrument, Australia), a ultraviolet–visible (UV–vis) detector (ICI 1200), and a data system for chromatogram integration (EZChrom, Scientific Software Inc., San Ramon, CA, USA), all operated at room temperature (24 ± 1°C). Separation was achieved on a Cosmosil 5C18 column (4.6 × 250 mm, particle size 5 μm, Kyoto, Japan). The mobile phase consisted of methanol–water (50:50, v/v, pH 3.0 adjusted by *ortho*-phosphoric acid) delivered at a flow rate of 1.0 ml min^{-1}. Tetramethylpyrazine was monitored at a wavelength of 280 nm throughout the experiments.

All calibration curves were required to have a correlation value of at least 0.995. Accuracy was calculated from the nominal concentration (C_{nom}) and the observed found concentration (C_{obs}). Precision CV (%) = [standard deviation (S.D.)/C_{obs}] × 100. Accuracy (%) = 100 ($C_{nom} - C_{obs}$)/C_{nom}. The same data used in the accuracy determinations were used for the calculation of precision. Intra-day and inter-day variabilities were determined by quantitating four replicates at concentrations of 0.01–5 μg ml^{-1} using the HPLC method described above on the same day and 4 different days, respectively.

The limit of detection (LOD) of the assay system was estimated as the tetramethylpyrazine quantity corresponding to three times the baseline noise. The limit of quantitation (LOQ) was defined as the lowest detectable quantity with deviation and precision of less than 15% (Causon, 1997).

Calibration curves were constructed based on HPLC analyses of various concentrations of te-

tramethylpyrazine in a range that covered the actual observed drug levels. The concentrations of tetramethylpyrazine in rat blood and brain dialysates were determined from the calibration curves. Absolute concentrations in extracellular fluid were calculated from the concentrations in the dialysates by the following equation: Concentration = (dialysate concentration/$R_{\text{in vivo}}$). Area under the concentrations versus time curve from time 0 to 2 h (AUC_{0-2}, in μg min ml^{-1}) for both blood and brain were determined using the trapezoidal rule. Blood brain barrier penetration ratios were determined by the brain-to-blood coefficient of distribution, $K_{\text{brain/blood}} = AUC_{\text{brain}}/AUC_{\text{blood}}$. For tetramethylpyrazine, the elimination half-life ($t_{1/2}$) was calculated using the Gauss–Newton algorithm by directly fitting the least-squares curve-fitting equation to the entire curve describing a two-exponential decay.

2.6. Statistical analysis

For comparison of the differences of pharmacokinetic parameters between blood and brain, *t*-tests for paired observations were performed. A *P* value of < 0.05 was considered the level of significance. All data are presented as mean ± S.E.M.

3. Results

Under the chromatographic conditions described, excellent separation and detection of tetramethylpyrazine were achieved in both blood and brain dialysates. The retention time of tetramethylpyrazine was found to be 7.3 min. The peak quantification limit of tetramethylpyrazine was 10 ng ml^{-1}. The standard curve, comprising standards at concentrations that covered the range of actual determinations in the experiments (0.01–5 μg ml^{-1}), was linear and could be established with acceptable accuracy (LOQ < 20%).

The reproducibility of the method was determined by examining both intra-day and inter-day variabilities. The intra-assay and inter-assay determination of tetramethylpyrazine at concentration ranges of 0.01–5 μg ml^{-1} were acceptable with precision and accuracy values of < 15%.

Table 1
In vivo recoveries of tetramethylpyrazine by microdialysis probes[a]

Concentration (μg ml^{-1})	Blood (%)	Brain (%)
0.1	58.97 ± 1.54	9.55 ± 1.01
1	60.60 ± 2.11	10.9 ± 1.37

[a] Data are expressed as mean ± S.E.M. ($n = 6$).

The in vivo microdialysate recoveries of tetramethylpyrazine in rat blood and brain based on 0.1 and 1 μg ml^{-1} standards are presented in Table 1. Based on the report by Telting-Diaz et al. (1992) that recovery is independent of both the concentration and the matrix (Ringer's solution, plasma or whole blood), subsequent probes were calibrated using only the Ringer's solution. Furthermore, since the process depends on simple diffusion, the rate of transfer across the membrane has been hypothesized to be equivalent at all concentrations, provided that other experimental conditions remain constant (Johansen et al., 1997).

The dialysate samples collected over the first 120 min were discarded to allow recovery from the acute effects of surgical trauma. The microdialysis–liquid chromatographic sampling system was then applied for the pharmacokinetic characterization and central nervous system distribution of tetramethylpyrazine. Fig. 1 shows the concentration versus time profiles of tetramethylpyrazine in the blood and brain after tetramethylpyrazine (10 mg kg^{-1}, i.v.) administration, the results having been corrected for in vivo probe recovery. The average brain/blood ratio of tetramethylpyrazine concentration continued to increase from 10 to 120 min following drug administration, suggesting continuing entry of tetramethylpyrazine into the brain. Other pharmacokinetic parameters are presented in Table 2.

4. Discussion

Although central nervous system activities can sometimes be affected by events in the peripheral system, most agents that affect central nervous

system functions, particularly those attributable to tetramethylpyrazine, viz. affecting local blood flow and cognitive functions, probably find their way into the brain and exert their effects locally.

Several techniques have been employed for the study of drug transport to the brain (Pardridge, 1998). Of these, the brain tissue homogenate technique is one of the traditional methods used to determine drug distribution in the brain. However, only single concentration–time points can be provided in such studies because the experimental animals are sacrificed for the determination. Thus to obtain a complete brain drug concentration–time profile, many animals must be used and inter-animal variability often compromises the accuracy of the results. Although they have the advantage of not being invasive, recently developed methods such as positron emission topography (PET) and nuclear magnetic resonance (NMR) require expensive materials as well as highly sophisticated and expensive equipment not commonly available in a general laboratory. On the other hand, microdialysis is relatively inexpensive and easy to set up. Custom-made probes further reduce expenses and allow customization for special needs such as sampling in blood. With certain precautions, such as allowing time for stabilization and partial recovery from surgical trauma and careful recovery calibration, the disadvantages can be minimized. The dialysates obtained can be analyzed using any appropriate analytical method. However, sensitivity of the method dictates the amount of dialysate required and therefore the sampling time, which is a disadvantage in that the values actually represent mean values over the period of dialysate collection. In our case, HPLC is again relatively accessible and the method has been optimized so that only 10 min of collection time is required.

Table 2
Pharmacokinetic analysis of tetramethylpyrazine in rat blood and brain[a]

Parameters	Blood	Brain
A (μg ml^{-1})	2.17 ± 0.47	4.15 ± 0.65*
B (μg ml^{-1})	1.12 ± 0.22	1.36 ± 0.17
α (1 min^{-1})	0.053 ± 0.004	0.049 ± 0.003
β (1 min^{-1})	0.027 ± 0.004	0.014 ± 0.002*
$t_{1/2,\alpha}$ (min)	14.4 ± 1.2	14.2 ± 0.8*
$t_{1/2,\beta}$ (min)	28.7 ± 4.1	52.9 ± 6.7*
AUC (μg min ml^{-1})	82.1 ± 14.9	184.6 ± 15.6*
Cl (1 kg^{-1} min^{-1})	0.15 ± 0.044	0.055 ± 0.005*

[a] Tetramethylpyrazine administration 10 mg kg^{-1}, i.v. Data are expressed as mean ± S.E.M. ($n = 4$). *, $P < 0.05$ vs. blood group.

The present results suggest that the pharmacokinetics of unbound tetramethylpyrazine appear to best fit the kinetics of a two-compartment model in both peripheral blood and central nervous systems. Analysis of the relationship between brain and blood levels, therefore, yields a progressively higher brain/blood ratio, suggesting appreciable blood–brain barrier penetrability. Such an observation is compatible with expectations for an agent possessing central nervous system activities (Watanabe, 1997). Ho et al. (1989) observed that tetramethylpyrazine can increase survival rate only if it is administered before the induction of cerebral ischemia. In addition, folk medicines often rely more on observations of clinical efficacy with relatively little support from basic data. The present study provides pharmacokinetic data in support of central action of tetramethylpyrazine.

In conclusion, our results show that it is feasible to simultaneously measure tetramethylpyrazine from blood and brain using two microdialysis probes. The microdialysis technique permits sampling of protein-free drug from blood and brain, which can be directly injected into a chromatographic system for continual in vivo monitoring. This method also has the advantage of causing minimal perturbances to the hemodynamics and normal physiological processes. The suggestion of appreciable blood–brain barrier penetrability supports the claim for central nervous system activities for tetramethylpyrazine.

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