Pharmacokinetic study of trimebutine maleate in rabbit blood using in vivo microdialysis coupled to capillary electrophoresis

Li Wang, Zhujun Zhang *, Weiping Yang

Department of Chemistry, Shaanxi Normal University, Xi'an 710062, China

Abstract

In vivo microdialysis was used together with capillary electrophoresis (CE) to monitor the concentration of trimebutine maleate (TM) in rabbit blood. Dialysis probe was perfused at 3 μl/min resulting in relative recovery of $26.6 \pm 3.1\%$ ($n = 3$). After a one step sample preparation the samples were injected directly into the capillary. TM was detected on-column using UV detector at 214 nm. Separation of TM from other components in the dialysate was achieved within 15 min. Evaluation was based on the relative collected peak height (TM/IS). The response for TM in the blood dialysate was linear over the range of 0.5–100 μg/ml. The detection limit of TM in the blood dialysate was 0.1 μg/ml (S/N = 3). This method has been successfully applied to the pharmacokinetic study of trimebutine maleate in rabbit blood following oral administration of 200 mg/kg. It provides a fast and simple technique for the pharmacokinetic study of TM in vivo.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Trimebutine; Microdialysis; Capillary electrophoresis; Pharmacokinetic

1. Introduction

Trimebutine maleate (TM), 2-dimethylamino-2-phenyl-butyl-3,4,5-trimethoxybenzoate hydrogen maleate (Fig. 1), is used in the treatment of various digestive tract disorders including dyspepsia, irritable bowel syndrome and post-operative ileus [1]. Various methodologies had been incorporated into high-performance liquid chromatography [2–6] or capillary electrophoresis assay [7] to detect TM in plasma. Generally, the protein-unbound form of drug was proposed as the active form of drug in the body. However, to obtain unbound drug levels to construct pharmacokinetic data, these methods have to be involved more complicated protein precipitation, extraction or derivatization procedures.

Recently, microdialysis had been extensively used to monitor the concentration of unbound drug in vivo, and the pharmacokinetic applications of this technique had been well covered in recent reviews [8–10]. This technique can in vivo measure endogenous and exogenous substances in extracellular fluid by implantation of a microdialysis probe in almost any organ or tissue of interest (brain, blood, bile, eye, etc.). It offers a number of important advantages over the classical approaches to carry out pharmacokinetic studies. Because there is no net fluid exchange, continuous sampling for long periods of time is possible, without interfering with the processes that govern the pharmacokinetic behavior of the drug. This results in drug microdialysate concentration–time curves with a high time resolution thus facilitating pharmacokinetic analysis. Since protein molecules are too large to pass though the dialysis membrane, microdialysis can directly measure protein-free drugs.

On the other hand, because the microdialysis sample available was small and the concentration of interesting drug in sample was very low, an analytical technique coupled with the microdialysis must have an effective separation and sensitive detection ability. Microdialysis samples have traditionally been analyzed by liquid chromatographic (LC) methods to gain resolution and quantification of the molecules of interest [11–14]. However, LC separations have

---

* Corresponding author. Tel.: +86 29 8530 8748; fax: +86 29 8530 7998.
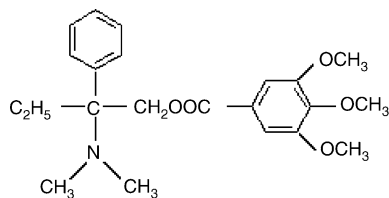*E-mail address:* zzj18@hotmail.com (Z. Zhang).

Fig. 1. Chemical structure of trimebutine (TM).

a relatively large injection volume requirement, as a consequence, which increases microdialysis sampling times. Capillary electrophoresis (CE), with its ability to resolve complex mixture efficiently and rapidly, and its low sample volume requirements, is becoming more popular as an alternative to LC [15,16].

In the present study, an in vivo microdialysis sampling method coupled with CE system was used to examine the free TM in rabbit blood. The limit of detection (S/N = 3) for TM in dialysate was $0.1 \, \mu g/ml$. In addition, the pharmacokinetics of free TM in rabbit blood following its oral (p.o.) administration 200 mg/kg was investigated utilized this sampling and assay system.

2. Experimental

2.1. Chemicals and reagents

TM was purchased from Sigma (St. Louis, MO). Verapamil hydrochloride (internal standard, IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The commercial TM tablets used were purchased from KaiKai Yuansheng Medicine Co. Ltd. (Hebei, China) containing 100 mg TM per tablet. Heparin sodium injection (12 500 IU) was obtained from Xuzhou First Biological Works (Xuzhou, China). The other chemicals were of analytical grade. Unless stated, all standards and solutions were prepared with dually distilled and de-ionized water.

2.2. Working solutions

Various TM working solutions in the range of $(0–2) \times 10^4 \, \mu g/ml$ were prepared by sequential dilution of TM stock solution (5 mg/ml). The $8 \, \mu g/ml$ IS working solution was obtained by diluting the IS stock solution (0.3 mg/ml).

The 30, 50, 100 mM Tris buffer adjusted to pH 2.5 with phosphoric acid; 50 mM sodium borate–sodium hydroxide buffer pH 10.0, 50 mM sodium phosphate–sodium hydroxide buffer pH 5.5 and 50 mM sodium acetate-acetic acid buffer pH 4.0 were prepared.

2.3. Equipment

Microdialysis sampling system was composed of a microinjection pump (Beijing Silugao High Technology

Development Co. Ltd., China), a 5 ml non-metallic gas tight syringe filled with water and microdialysis probes (10 000 D cut-off) obtained from Xi'an Kangpei New Technology Co. (Xi'an, China). A 60 cm long PTFE tubing of 0.25 mm i.d. and 0.8 mm o.d. was used for all connections.

The CE device consisted of a Beckman System P/ACE 5000 with a UV-absorbance detector using a filter of 214 nm. Data were collected using the Beckman P/ACE Station software system (Version 1.21). An uncoated fused-silica capillary of 47 cm (detection length 40 cm) × 50 μm i.d. was used. Both of these were purchased from Beckman (Beckman Coulter, Fullerton, CA, USA). The capillary was pre-rinsed with water (2 min) and separation buffer (2 min); before each electrophoretic run, the capillary was sequentially washed with 0.1 M NaOH (2 min), water (2 min) and separation buffer (2 min). Samples were loaded by pressure injection at 3.45 kPa (0.5 p.s.i.) for 10 s, for a total volume of about 9 nl and were separated at an applied voltage of 18 kV. Running electrolytes were filtered through a 0.45 μm nylon membrane, and degassed before use. The temperature was held at 25 °C.

2.4. Microdialysis probe calibration

In order to ascertain the in vivo concentration of TM, it was necessary to determine the recovery of the dialysis probe. For pharmacokinetic study, the in vivo recovery was calculated by retrograde method usually [17,18]. However, in this work, the perfusion fluid was water so a retrograde calibration technique could not be used. In blood, in vitro calibration can result in accurate recovery values [10]. Then the in vitro recovery of microdialysis probe was studied in this work. This was accomplished by placing the probe in standard TM solutions (30, 40, 50 μg/ml) prepared in Ringer's solution that was maintained at 37 °C in a water bath to mimic the rabbit body temperature. The flow-rate was 3 μl/min, the same as that used for pharmacokinetic studies. For each TM concentration, the dialysate collection began after an equilibration time of 30 min. For every change of TM concentration, the first dialysate was discarded to avoid the residual effect of the previous concentration. The collection interval was 15 min to yield about 45 μl of sample. An amount of 20 μl microdialysis sample was spiked with 5 μl of IS working solution, and this mixture was injected to the CE system. Recovery by the probe was calculated from the concentration in the dialysate (C_{out}) divided by the concentration of the spiked drug in the test tube (C_{in}), that is, $\text{Recovery}_{in \, vitro} = [C_{out}/C_{in}] \times 100$. The results are given in Table 1. These results showed that the in vitro recovery of the probe was independent of TM concentration. The average recovery for TM was $26.6 \pm 3.1\%$ ($n = 3$).

2.5. In vivo pharmacokinetic experiments

Five male rabbits were used. Using the system shown in Fig. 2, the determination of TM in the edge vein of rabbit's
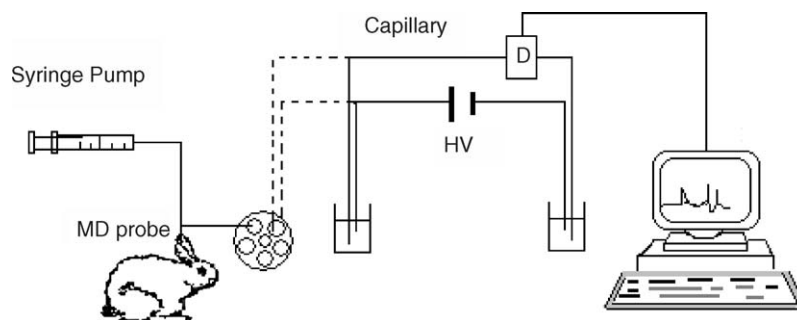
Fig. 2. Schematic diagram of microdialysis–CE system.

Table 1
In vitro microdialysis probe recoveries (%)[a]

TM concentration (μg/ml)	Recovery (%)
30	29.9 ± 3.5
40	23.5 ± 3.5
50	26.4 ± 2.2
Average	26.6 ± 3.1

[a] Date are expressed as mean $\pm$ S.D.

ear was carried out. Each microdialysis probe was perfused with degassed water for at least 40 min prior to use. A male rabbit (approximate 2.5 kg), without anaesthetization, was fixed on a wooden dissecting plate. About 20 min before the experiments, the rabbit was given an intravenous injection of 5000 IU of heparin sodium injection via the edge vein of the rabbit's ear/right to avoid clogging by blood coagulation during the experiments. The microdialysis probe was inserted into the edge vein of the ear/left, and then was continuously perfused. Blanks were collected immediately following insertion of the microdialysis probe. A 30 min period was allowed following probe insertion, in order to allow the whole system to stabilize, after which time the rabbit was p.o. dosed with TM (200 mg/kg). Dialysis samples were collected at 15 min intervals. The remaining procedure was same as described in the Section 2.4 until TM was no longer detected in the dialysate.

3. Results and discussions

3.1. Microdialysis experiments

In microdialysis, the perfusion fluid often was Ringer's solution or 0.9% (w/v) physiological saline solution in order to match the perfusion fluid as closely as possible to the ionic strength and composition of the tissue interstitial fluid [19,20]. This is done to prevent possible osmotic pressure effects and loss of fluid from either the perfusion fluid or the tissue interstitial fluid [21]. However, the small amount of sample that can be loaded onto the capillary, the anti-stacking exhibited by high ionic strength samples [14] and the low sensitivity of UV detection [16] limited the application of CE-UV system for microdialysate analysis. In order to improve

the sensitivity, several methods have been used [22,23] such as, derivation dilysates with other agents [22] and dilution dilysates with water or other non-ionic solvents [23]. But the procedures often were tedious or LOQ often was high. In this work, to improve the sensitivity of the method, Ringer's solution, physiological saline solution and water as perfusion fluid were studied. Since perfusion with water showed a higher response compared to Ringer's solution and physiological saline solution, water was selected as perfusion fluid. A perfusion rate of 3 μl/min was selected as a compromise between the sensitivity and the separation time for subsequent investigations.

3.2. Capillary electrophoresis

When performing microdialysis, there are many interferences that are present in the dialysate. Therefore, the migration behavior of the blank dialysate was investigated.

Several variables were investigated, including the pH and composition of the run buffer and the applied voltage. Of these, pH and composition of run buffer were found to have the greatest effects on the separation. At borate buffer pH 10.0, both TM and IS are anionic and the electroosmotic flow is very fast, resulting in poor resolution. Phosphate buffer was investigated at lower pH value 5.5, but no good separation was obtained. Acetate buffer pH 4.0 and Tris buffer pH 2.5 were also investigated. A Tris buffer, pH 2.5 provided the best resolution.

The results also showed that the use of higher concentrations of run buffer and lower applied voltages improved resolution. However, these conditions resulted in longer analysis times. In addition, the use of high concentrations of buffer resulted in high separation currents and increased Joule heating. Considering all of these factors, the best separation was obtained under the following conditions: 50 mM Tris–H$_3$PO$_4$ buffer, pH 2.5, 18 kV applied voltage.

Fig. 3 shows a typical electropherogram of a standard mixture of TM and IS. Fig. 4 shows the electropherograms of (a) a blank blood microdialysis sample and (b) a sample spiked with IS and TM. It can be seen that under these conditions, there were no observed peaks that would significantly interfere with the determination of TM. In this case, the migration time for TM was about 8.6 min, IS was
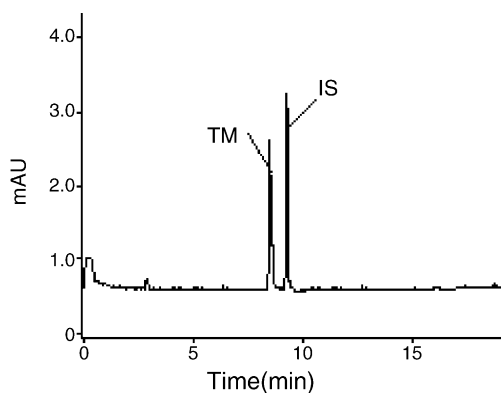
Fig. 3. Typical electropherogram of TM and IS mixture. CE run buffer, 50 mM Tris–H$_3$PO$_4$, pH 2.5; separation voltage, 18 kV; separation capillary, 47 cm × 50 μm i.d.

about 9.3 min and the overall analysis time was less than 15 min.

3.3. Method validation

Calibration samples were obtained by spiking 19 parts of blank microdialysate with one part of corresponding working solutions to yield several concentrations in the range of 0.1–100 μg/ml. The perfusion media and pumping flow-rate were the same as described above. All samples were processed further as described in Section 2.5 and analyzed
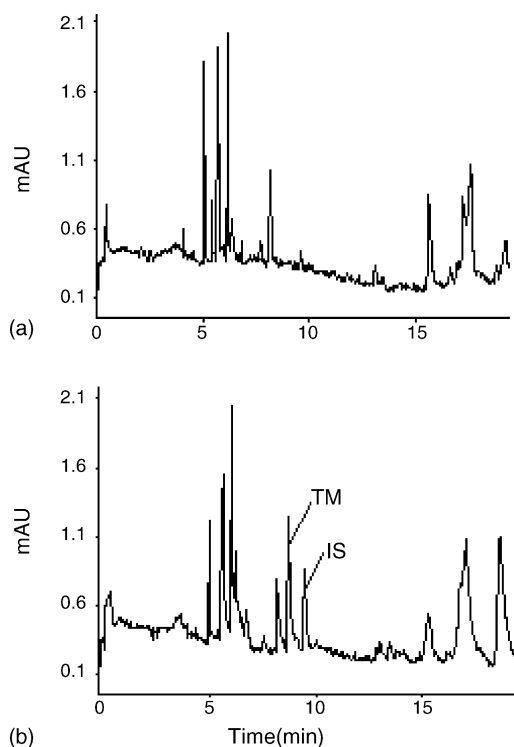

Fig. 4. Typical electropherograms of (a) blank blood dialysate, (b) blood dialysate spiked with IS and TM. CE run buffer, 50 mM Tris–H$_3$PO$_4$, pH 2.5; separation voltage, 18 kV; separation capillary, 47 cm × 50 μm i.d.; injection time, 10 s.

Table 2
Intra- and inter-day accuracy and precision of the determination of TM in microdialysate

Nominal concentration (μg/ml)	Observed concentration (μg/ml)[a]	%RSD	Accuracy (%Bias)
Intra-assay ($n = 3$)			
0.50	0.45 ± 0.061	13.5	−10
10.0	10.5 ± 0.61	5.8	5
20.0	19.6 ± 0.61	3.1	−2
50	48 ± 1.7	3.5	−4
100	106 ± 4.3	4.1	6
Inter-assay ($n = 3$)			
0.50	0.56 ± 0.081	14.5	12
10.0	10.2 ± 0.46	4.5	2
20.0	19.3 ± 0.62	3.2	−3.5
50	52 ± 1.6	3.1	4
100	98 ± 3.4	3.5	−2

[a] Observed concentrations date are expressed as mean ± S.D.

in one sequence. Calibration curve according to the internal standard method was obtained by plotting concentration versus corrected peak height. The results showed that the peak ratios (TM/IS) were linear to TM concentrations ranging from 0.5 to 100 μg/ml. Typical equation of calibration curve is $Y = 0.4973X − 0.1597$ ($r > 0.996$).

The intra-day and inter-day variabilities of TM were assayed (three replicates) at concentrations of 0.5–100 μg/ml on the same day and on three sequential days, respectively. The accuracy (%Bias) was calculated from the nominal concentrations (C_{nom}) and the mean value of observed concentrations (C_{obs}) as follows: %Bias = $[(C_{obs} − C_{nom})/(C_{nom})] × 100$. The precision coefficient of variation (RSD) was calculated from the observed concentrations as follows: %RSD = [standard deviation (S.D.)/C_{obs}] × 100. Accuracy (%Bias) and precision (%RSD) values of ±15%, covering the range of actual experimental concentrations, were considered to be acceptable [24]. Intra- and inter-day precision and accuracy for TM (Table 2) fell well within predefined limits of acceptability. The limit of quantitation (LOQ) for TM in dialysate was set at the concentration of the lowest non-zero calibration standard (S/N ≥ 10:1). The limit of detection (LOD) of TM in dialysate was determined as a signal to noise baseline ratio of 3:1. The LOD was found to be 0.1 μg/ml and the LOQ was 0.5 μg/ml.

3.4. Date analysis

Concentrations of TM in rabbit blood dialysates were determined from calibration curve. The free concentration of TM in blood (C_{blood}) was calculated from its concentration in the dialysate ($C_{dialysate}$) by the following equation: $C_{blood} = [C_{dialysate}/Recovery] × 100$. Initially, the amount of TM in the dialysate samples increased, with maximal concentration of 202 μg/ml reached at 60 min. The concentration of TM in the dialysates rapidly decreased as shown in Fig. 5.

Pharmacokinetic parameters were calculated by the NDST-21 software (China Pharmaceutical University,
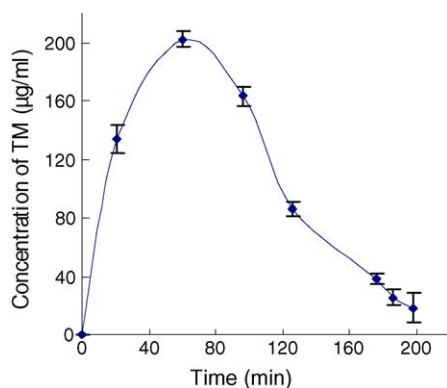
Fig. 5. Mean protein-unbound blood concentration–time profile after TM administration (200 mg/kg, p.o. Data are expressed as means ± S.E.M. $n = 5$).

Table 3
Pharmacokinetic parameters for TM[a]

Parameters	Estimate
$T_{1/2}$Ka (min)	25.0 ± 5.3
$T_{1/2}$Ke (min)	31.9 ± 6.6
CL (ml/(kg min))	7.7 ± 2.3
AUC$_{0-\infty}$ (µg min/ml)	26264.0 ± 201
AUC$_{0-T}$ (µg min/ml)	23192.7 ± 198
C_{max} (µg/ml)	202 ± 14
T_{max} (min)	60 ± 3

[a] Date are expressed as mean ± S.E.M. ($n = 5$).

China). The areas under the plasma concentration–time curves (AUC$_{0-t}$) were calculated using the trapezoid method. The AUC$_{0-\infty}$ were calculated by the trapezoidal rule and extrapolated to time infinity by the addition of AUC$_{t-\infty}$. Half-life ($T_{1/2}$) values were calculated using the equation: $T_{1/2}$Ke = 0.693/β for elimination half-life; $T_{1/2}$Ka = 0.693/α for distribution half-life. Where α and β are the distribution and elimination rate constants, respectively. The clearance (CL) was calculated as: CL = dose/AUC$_{0-\infty}$. The results were shown in Table 3.

4. Conclusions

A routine CE assay to measure TM in blood dialysate was established successfully. The detection limit for TM in dialysate was 0.1 µg/ml. The use of UV detection at 214 nm provided adequate sensitivity for the detection of the drug in blood microdialysate. This method was used to monitor the pharmacokinetics of TM following the p.o. dose of

200 mg/kg of TM to rabbits. The separation was fast and efficient. Therefore the method is suitable for situations with minimal matrix volumes: e.g. pediatrics, patients at risk, animal-, microdialysis- and tissue-kinetic studies.

Acknowledgement

References

[1] V. Kayser, D. Christensen, G. Guilbaud, F. Roman, Life Sci. 66 (2000) 433–439.
[2] H. Wang, H. Zhou, S. Horimoto, J. Jiang, T. Mayumi, P. Hu, J. Chromatogr. B 779 (2002) 173–187.
[3] E.-H. Joo, W.-I. Chang, I. Oh, S.-C. Shin, H.-K. Na, Y.-B. Lee, J. Chromatogr. B 723 (1999) 239–246.
[4] A. El-Gindy, S. Emara, G.M. Hadad, J. Pharm. Biomed. Anal. 33 (2003) 231–241.
[5] F. He, J.S. Tu, L.L. Yin, Y.L. Hu, J.S. Zhang, J. Chin. Med. Univ. 32 (2001) 122–126.
[6] H. Jiang, L. Ding, J. Yang, X. Huang, G.Y. Liu, Z.X. Zhang, Acta Pharm. Sinica 39 (2004) 208–211.
[7] L.N. Yu, F.M. Li, Acta Pharma. Sinica 36 (2001) 131–133.
[8] R.K. Verbeeck, Adv. Drug Deliv. Rev. 45 (2000) 217–228.
[9] T.-H. Tsai, J. Chromatogr. B 797 (2003) 161–173.
[10] D.J. Weiss, C.E. Lunte, S.M. Lunte, Trends Anal. Chem. 19 (2000) 606–616.
[11] L. Lai, L.-C. Lin, J.-H. Lin, T.-H. Tsai, J. Chromatogr. A 987 (2003) 367–374.
[12] T.-H. Tsai, M.-C. Liu, J. Chromatogr. B 806 (2004) 161–166.
[13] M.A. Gilinsky, A.A. Faibushevish, C.E. Lunte, J. Pharm. Biomed. Anal. 24 (2001) 929–935.
[14] M.I. Davies, J.D. Cooper, S.S. Desmond, C.E. Lunte, S.M. Lunte, Adv. Drug Deliv. Rev. 45 (2000) 169–188.
[15] J. Zhou, D.M. Heckert, H. Zuo, C.E. Lunte, S.M. Lunte, Anal. Chim. Acta 379 (1999) 307–317.
[16] L.A. Dawson, J. Chromatogr. B 697 (1997) 89–99.
[17] G. Stagni, C. Shukla, J. Controlled Release 93 (2003) 283–292.
[18] T.-H. Tsai, J.-W. Wu, Anal. Chim. Acta 448 (2001) 195–199.
[19] E.C.M. de Lange, M. Danhof, A.G. de Boer, D.D. Breimer, Brain Res. Rev. 25 (1997) 27–49.
[20] E.C.M. de Lange, A.G. de Boer, D.D. Breimer, Adv. Drug Deliv. Rev. 45 (2000) 125–148.
[21] J.A. Stenken, Anal. Chim. Acta 379 (1999) 337–358.
[22] T. Hu, H. Zou, C.M. Riley, J.F. Stobaugh, S.M. Lunte, J. Chromatogr. A 716 (1995) 381–388.
[23] B.X. Mayer, U. Hollenstein, M. Brunner, H.-G. Eichler, M. Müller, Electrophoresis 21 (2000) 1558–1564.
[24] R. Causon, J. Chromatogr. B 689 (1997) 175–180.