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Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1453–1457
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

In vivo and real time determination of ornidazole and tinidazole and pharmacokinetic study by capillary electrophoresis with microdialysis

Liyuan Zhang, Zhujun Zhang *, Ke Wu

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

Received 3 January 2006; received in revised form 12 March 2006; accepted 18 March 2006

Abstract

The aim of this study was to develop a rapid and sensitive method for in vivo and real time monitoring unbound ornidazole (ONZ) and tinidazole (TNZ) in rabbit blood using capillary electrophoresis coupled with microdialysis. The UV wavelength was set at 214 nm and all separations were performed in 20 mM Tris–H_3PO_4 (pH 1.5) buffer. Microdialysis probes were perfused at 4 μl/min resulting in relative recoveries of 33.1 ± 3.6% and 34.8 ± 3.3% ($n = 3$) for ONZ and TNZ, respectively. The linearity was studied in the concentration range of 1.0–412 μg/ml for ONZ and 1.0–520 μg/ml for TNZ. The detection limits were 0.7 μg/ml for ONZ and 0.6 μg/ml for TNZ ($S/N = 3$). All separation could be achieved within 15 min. This method has been successfully applied to the pharmacokinetic study of ONZ and TNZ in rabbit blood.

Keywords: Ornidazole; Tinidazole; Microdialysis; Capillary electrophoresis; Pharmacokinetic

1. Introduction

Ornidazole (ONZ) (1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole) and tinidazole (TNZ) (1-(2)-(ethylsulfonyl)ethyl)-2 methyl-5-nitroimidazole) (Fig. 1) both are members of the 5-nitroimidazole group. They have a heterocyclic structure consisting of an imidazole-based nucleus with a nitro group, NO_2, in position 5. They have become a well-established group of antiprotozoan and antibacterial agents. Owing to their bactericidal and antiprotozoal activity, these chemotherapeutic agents inhibit the growth of both anaerobic bacteria and certain anaerobic protozoa, such as *Trichomonas vaginalis*, *Entamoeba histolytica* and *Giardia lamblia*. They can also be used effectively against metronidazole-resistant strains of *Trichomonas vaginalis*. They have a greater tolerability, prolonged half-life and a more favorable side-effect profile than metronidazole [1,2]. A few papers have reported determination methods for ONZ and TNZ. These include electrochemical methods [3,4], high performance thin layer chromatography [5,6], high performance liquid chromatography [7,8], and a UV spectrophotometric method [9,10]. However, nearly all of these methods reported could not monitor drug concentration in blood on-line, real time and in vivo.

Microdialysis had been extensively used recently to monitor the concentration of unbound drug in vivo and to study drug's pharmacokinetics [11,12]. This technique offers a number of important advantages to investigate the pharmacokinetic studies. Because there is minimal perturbation to tissues and organs due to the small size of the probe, and no change to the fluid volume of the surroundings, this process enables the long-term continuous sampling without interfering with the processes that govern the pharmacokinetic behavior of the drug. The methods commonly used to couple with microdialysis are LC [13,14] and CE [15,16]. However, LC requires a relatively large sample volume, which increases microdialysis sampling time. Capillary electrophoresis (CE), requires less sample volume and so offers an important advantage over LC.

The objective of this work was to develop a CE method for the determination of ONZ and TNZ in rabbit on-line, real time, and in vivo. A particular emphasis was placed on the investigation of the pharmacokinetic features of ONZ and TNZ.

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

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doi:10.1016/j.jpba.2006.03.016

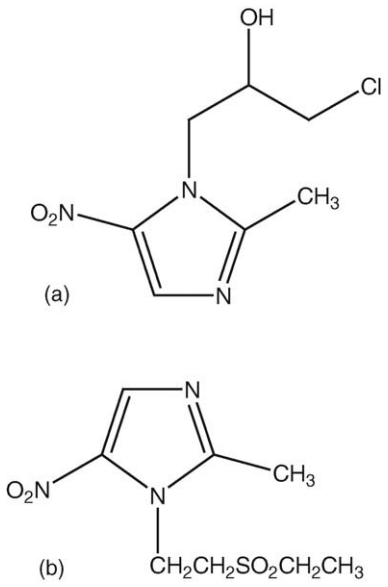

Fig. 1. Chemical structure of ONZ (a) and TNZ (b).

2. Experiment

2.1. Chemicals and reagents

ONZ and TNZ were purchased from Sigma (St. Louis, MO). The commercial ONZ capsules used were purchased from Wanlong Medicine Co. Ltd. (Xi'an, China) containing 250 mg ONZ per capsule. The TNZ and glucose injection was purchased from Kelun Medicine Co. Ltd. (Sichuan, China) containing 400 mg TNZ and 5 g glucose per 100 ml solution. 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. Stock solutions of ONZ and TNZ were prepared to 1 and 4 mg/ml and then diluted to desired concentrations.

2.2. Equipment

The microdialysis sampling system was composed of a microinjection pump (Beijing Silugao High Technology Development Co. Ltd., China), a 5 ml plastic gas tight syringe and microdialysis probes (10,000 D cut-off) obtained from Xi'an Kangpei New Technology Co. (Xi'an, China). A 60-cm long section of PTFE tubing of 0.25 mm i.d. and 0.8 mm o.d. was used for all connections. All determinations were performed on a P/ACE 5000 system (Beckman Coulter Instrument, Fullerton, CA, USA) equipped with a UV detector with the wavelength set at 214 nm. The system was controlled by P/ACE Station (Version 1.21) software. The separation was carried out on a 47 cm (40 cm to the detector) × 50 μm i.d. fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China). The capillary was treated prior to its first use by flushing with 1.0 M HCl for 20 min, 0.2 M NaOH for 20 min and distilled water for 10 min. Between two runs, a rinse-cycle, 0.1 M HCl, distilled water 0.1 M NaOH, distilled water and run buffer each for 2 min were used. The capillary was maintained at 25 °C. Sample was injected by applying a pressure of 3.45 kPa (0.5 psi) for 14 s and the injection volume was about 16.8 nl.

2.3. Microdialysis probe recovery

A retrograde calibration technique was used for in vivo recovery determinations [17,18]. To investigate the microdialysis probe recovery for ONZ, TNZ was used as the IS. The particular operations were described as follows: the microdialysis probe was inserted into the edge vein of a rabbit ear and perfused with Ringer's solution [19–21] containing TNZ (10, 20, and 40 μg/ml) at a flow rate of 4 μl/min. The temperature of the solution was maintained at 37 °C to mimic the rabbit body temperature. Recovery was calculated as: $\text{recovery}_{\text{in vivo}} = 1 - (C_{\text{perfusate}}/C_{\text{dialysate}})$, where $C_{\text{perfusate}}$ is the concentration of TNZ in perfusing solution and $C_{\text{dialysate}}$ is the TNZ concentration in dialysate. The dialysate collection began after an equilibration time of 60 min, and with a collection interval of 15 min to yield about 50 μl of solution from each sample. A 30 μl of dialysate was assayed using the capillary electrophoresis. The measurement of microdialysis probe recovery for TNZ was similar to ONZ, with the IS turned to ONZ. Microdialysis probe recoveries for ONZ and TNZ are showed in Table 1.

2.4. Pharmacokinetic study

The experiments were performed on five healthy male rabbits weighing 2–2.5 kg. The rabbits were housed under standard laboratory conditions and fed with normal rabbit feed and provided with regular drinking water. At the time when the experiments were performed, the rabbit's age ranged from 5 to 6 months. Microdialysis sampling has been coupled on-line to CE for pharmacokinetic investigations in awake, freely-moving rabbit. About 30 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 ear to avoid clogging by blood coagulation during the experiments and then the microdialysis probe was inserted into the edge vein of the other ear. Microdialysis probe was perfused with Ringer's solution at a flow rate of 4 μl/min using a microinjection pump. Blank dialysates were monitored for at least 1 h prior to administration of the

Table 1
In vivo microdialysis probe recoveries[a] (%) of ONZ and TNZ in rabbit blood ($n = 3$)

Microdialysis probe recoveries for ONZ		Microdialysis probe recoveries for TNZ	
TNZ concentration (μg/ml)	Recovery (%)	ONZ concentration (μg/ml)	Recovery (%)
10	35.8 ± 3.9	10	35.5 ± 4.3
20	32.3 ± 2.2	20	31.6 ± 2.9
40	36.3 ± 3.7	40	32.3 ± 3.5
Average	34.8 ± 3.3	Average	33.1 ± 3.6

[a] Data are expressed as mean ± S.D.

ONZ capsules 100 mg/kg or intravenous administration of TNZ 50 mg/kg. This equilibration period is necessary since it has been reported that probe introduction is associated with pathophysiological changes in the blood, such as an increase in blood flow [22]. Dialysate samples were continuously collected and injected into the CE analysis system until ONZ or TNZ was no longer detected in the dialysate.

3. Results and discussions

3.1. Capillary electrophoresis

In this work, various parameters of separation, such as the type, pH and concentration of run buffer, applied voltage and injection time were investigated through testing the migration behavior of ONZ and TNZ to achieve optimal separation, the highest sensitivity and the shortest analysis time. It was observed that the separation between ONZ and TNZ could not be obtained when the buffer pH was higher than 4.0. Although separation was achieved at amino acetic acid buffer pH 2.4, the sensitivity was much lower than Tris buffer. Besides, the use of applied high voltage resulted in high separation currents and increased Joule heating. Above all, considering all of these factors, the best separation conditions were as follows: 20 mM Tris–H_3PO_4 buffer; pH 1.5; 15 kV applied voltage.

Fig. 2 shows the typical electropherograms of: (a) a blood dialysate sample after oral administration ONZ and (b) a sample after intravenous TNZ. It can be seen that under these conditions, there were no observed peaks that would significantly interfere with the determination of ONZ or TNZ. In this case, ONZ and TNZ appeared at average migration time (R.S.D.%) of 9.87 min (1.1%) and 10.53 min (1.5%), respectively. The overall analysis time was less than 15 min.

3.2. Linearity and R.S.D.

Under the optimum analysis conditions, linearity was studied in the concentration range of 1.0–412 μg/ml for ONZ and 1.0–520 μg/ml for TNZ, when the microdialysis probe was perfused with Ringer's solution at the rate of 4 μl/min. During the investigation of ONZ, 20 μg/ml TNZ was used as an internal standard, in the same way, in the study of TNZ, 20 μg/ml ONZ was used as the internal standard. The peak area ratios of ONZ and TNZ to its IS were plotted versus the concentrations of the calibration standards. The linearity equations were $Y = 0.02747 + 0.03057c$ ($R = 0.9995$) for ONZ and $Y = 2.965 + 0.03394c$ ($R = 0.9996$) for TNZ, where Y is the ratio of peak normalization and c is the concentration expressed in μg/ml ($n = 9$). The detection limits were 0.7 μg/ml for ONZ and 0.6 μg/ml for TNZ at a signal-to-noise ratio of 3. The intra-day and inter-day variabilities of the assay method were determined by repeated analysis of three quality control samples on the same day and on five different days, respectively. The results are shown in Table 2. This data indicates that the assay method is precise within the same day and across multiple days [23].

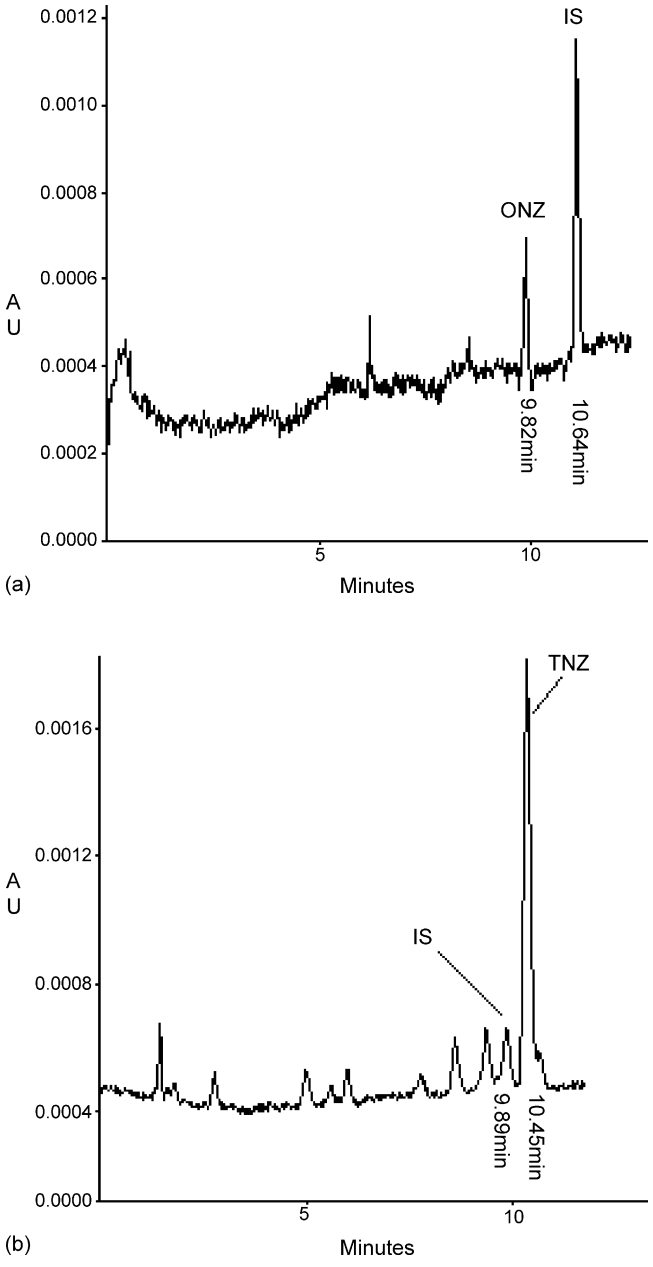

Fig. 2. Typical electropherograms of blood dialysate: (a) after oral administration ONZ and (b) after intravenous administration TNZ.

3.3. Data analysis

Concentrations of ONZ or TNZ in rabbit blood dialysate were determined from calibration curve. The free concentration of ONZ or TNZ in blood (C_{blood}) was calculated from its concentration in the dialysate ($C_{dialysate}$) by the following equation: $C_{blood} = [C_{dialysate}/\text{recovery}] \times 100$. Initially, the amount of ONZ in the dialysate samples increased, with maximal concentration of 24 μg/ml reached at 2 h and then decreased slowly, as shown in Fig. 3(a). Similarly, the concentrating of TNZ in the dialysate samples reached maximum of 51.4 μg/ml at 1.5 h and then decreased rapidly, as shown in Fig. 3(b).

Pharmacokinetic parameters were calculated by the Drug and Statistics Ver1.0 software (The Center for Drug Evaluation of

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

Normal concentration (μg/ml)	Intra-day precision and accuracy ($n=5$)			Inter-day precision and accuracy ($n=5$)		
	Observed concentration (μg/ml)[a]	R.S.D. (%)	Accuracy (%bias)	Observed concentration (μg/ml)[a]	R.S.D. (%)	Accuracy (%bias)
ONZ						
1	1.07 ± 0.095	8.9	7.0	1.13 ± 0.11	9.7	13
200	208 ± 8.7	4.2	4.0	205 ± 10.5	5.1	2.5
412	409 ± 13	3.2	−0.73	408 ± 8.3	2.1	−0.97
TNZ						
1	1.03 ± 0.037	3.6	3	0.94 ± 0.054	5.7	−6
260	261.7 ± 3.4	1.3	0.65	264.2 ± 7.7	2.9	1.6
520	515 ± 9.8	1.9	−0.96	522.6 ± 13.1	2.5	0.5

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

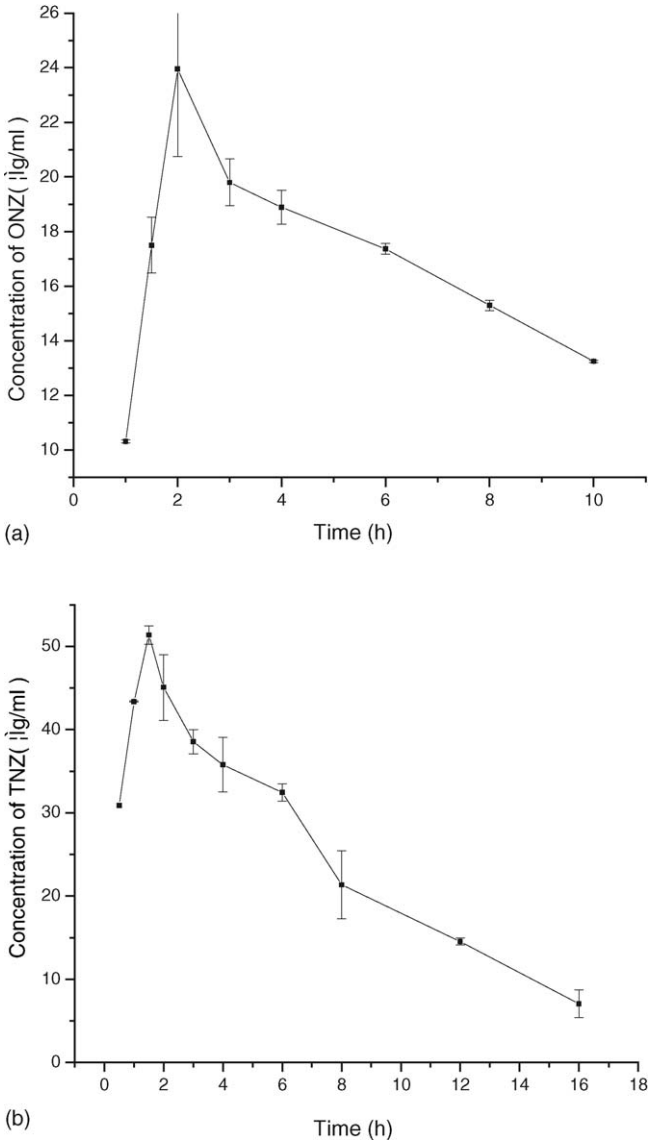

Fig. 3. Mean concentration–time curve of ONZ (a) and TNZ (b).

Table 3
Pharmacokinetic parameters for ONZ and TNZ[a]

Parameters	Estimate	
	ONZ	TNZ
$T_{1/2}Ka$ (h)	0.311 ± 0.0622	0.347 ± 0.12
$T_{1/2}Ke$ (h)	10.823 ± 2.39	5.276 ± 1.34
CL (L/kg/h)	0.269 ± 0.0534	0.114 ± 0.0137
$AUC_{0-\infty}$ (μg h/ml)	338.1 ± 55.5	439.8 ± 27.6
AUC_{0-T} (μg h/ml)	92.7 ± 57.3	378.84 ± 29.3
C_{max} (μg/ml)	23.9 ± 3.5	51.36 ± 3.8
T_{max} (h)	2.000 ± 0.429	1.500 ± 0.237

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

China, 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: $T_{1/2}Ke = 0.693/\beta$ for elimination half-life; $T_{1/2}Ka = 0.693/\alpha$ 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 are shown in Table 3. Based on pharmacokinetic calculation, ONZ and TNZ both best fitted to one-compartmental model.

4. Conclusion

A simple, fast and reliable CE method was developed successfully for the measurement of free ONZ and TNZ in rabbit blood. The method presents a good performance with respect to specificity, linearity, accuracy, precision and robustness.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (Grant No. 30470886).

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