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Determination and pharmacokinetics of ergometrine maleate in rabbit blood with on line microdialysis sampling and fluorescence detection

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Abstract

The study describes a flow injection on-line microdialysis system for in vivo monitoring of ergometrine maleate in rabbit blood with fluorescence detection. A flow-through microdialysis probe was used for intravenous sampling by pumping of the blood from the tested rabbit through the flow-through microdialysis probe located outside the living system at a flow rate of 15 μl min^{-1}. The perfusion rate is 5 μl min^{-1}. The ergometrine maleate in the dialysate was detected on-line with a flow injection fluorescence system after the ergometrine maleate administration (0.2 mg kg^{-1}, i.v.). The dialysate sample volume was about 15 μl. The system was linearly related to the concentration of ergometrine maleate in the range 1–140 ng ml^{-1} ($r = 0.9989$) with a detection limit 0.3 ng ml^{-1} (3σ). The pharmacokinetic parameters of ergometrine maleate were calculated utilizing the pharmacokinetic software 'NDST-21' by a one-compartmental open model.

Keywords: Ergometrine maleate; Microdialysis; In vivo; Fluorescence; Pharmacokinetics

1. Introduction

In recent years, microdialysis sampling has become a well-known technique for in vivo monitoring of biochemical constitutes in the extracellular fluid (ECF) of virtually any tissue, organ or biological fluid [1]. While microdialysis sampling was originally developed mainly to monitor neurotransmitter release in the brain [2], over the past decade the technique has been employed extensively for in vivo analysis at other sites of living systems, such as muscle [3], liver [4,5], bile [6], skin [7,8], tumor [9], blood and brain [10–13]. Furthermore, the application of microdialysis sampling has been extended to many other fields including toxicology [4], bioprocess monitoring [14] and pharmacokinetics [11–13].

The flow-through microdialysis probe, originally introduced by Fang et al. [15], enables the monitoring of the concentration of glucose in the blood of rabbit by pumping of the blood from the tested rabbit through the microdialysis probe located outside the living system. A diagram of the flow-through microdialyzer is shown in Fig. 2. If the microdialysis probe is implanted in a blood stream, the disadvantages of microdialysis system are obvious. The variations in blood flow always somewhat affect analyte transfer through dialysis membranes [16], furthermore, irreproducible partial obstruction of membrane surface of a dialysis probe by the vein walls during implantation and/or during the monitoring processes cannot be completely avoided [15]. However, those disadvantages can be avoided by the flow-through microdialysis probe. In recent years, flow-injection analysis (FIA) is a widely used methodology to perform the automation of analytical progress in many fields so several works dealing with FIA combined with microdialysis have appeared in the literatures [15,17–19].

Ergometrine maleate ((8s)-9,10-didehydro-*N*-[(s)-2-hydroxy-1-methylethyl]-6-methylergoline-8-carboxamide mon-

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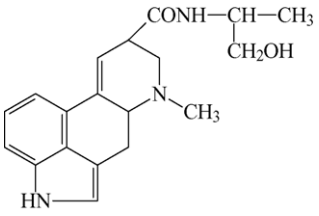

Fig. 1. Structure of ergometrine.

omaleate) is a naturally occurring amino ergot alkaloid, used as an orally active obstetric drug and it has a direct stimulating action on the vascular smooth muscle. Several methods have been reported for the determination of ergometrine maleate such as HPLC [20], spectrophotometry [21], immunoassay [22,23], amperometry [24], and chemiluminescence [25]. Fluorescent detection is widely used in quantitative analysis because of its great sensitivity and good selectivity as well as its relatively low cost. Ergometrine (Fig. 1) has a property of fluorescence [26], however, few reports describe the microdialysis sampling method coupled with fluorescent detection for determination of ergometrine maleate.

Therefore, in the present study we use an in vivo on-line microdialysis sampling method coupled with flow-injection analysis fluorescence detection for the measurement of ergometrine maleate in the rabbit blood. Furthermore, the pharmacokinetic analysis of results were obtained utilizing software 'NDST-21'.

2. Experimental

2.1. Reagents and standard solutions

All chemicals were of analytical reagent grade, and doubly distilled water was used throughout. The Ringer's solution containing 148 mM NaCl, 4.0 mM KCl and 2.3 mM $CaCl_2$ used as perfusion medium was prepared by dissolving 8.65 g of NaCl and 0.30 g of KCl and 0.26 g of $CaCl_2$ in 1 l of water. Ergometrine maleate was purchased from Harvest Pharmaceutical Corporation (Shanghai, China). Aqueous ergometrine maleate standards were prepared by sequential dilution of a stock solution containing 10 μg ml^{-1} ergometrine maleate with perfusion solution.

2.2. Instrumentation

Microdialysis sampling was performed using a KH-1 microdialysis syringe pump controller (Institute of Chemistry Academy of Sciences, China) coupled to a flow-through microdialysis probe. A microdialysis syringe pump (1000 μl volume) was used for delivery of perfusate. A variable peristaltic pump (Xi'an Ruimai Instrumental Factory, Xi'an, China) was used for pumping the blood from the tested rabbit. PTFE tubing of (0.25 mm i.d., 0.8 mm o.d.) was used for all connections. The microdialysis manifold is shown in Fig. 2.

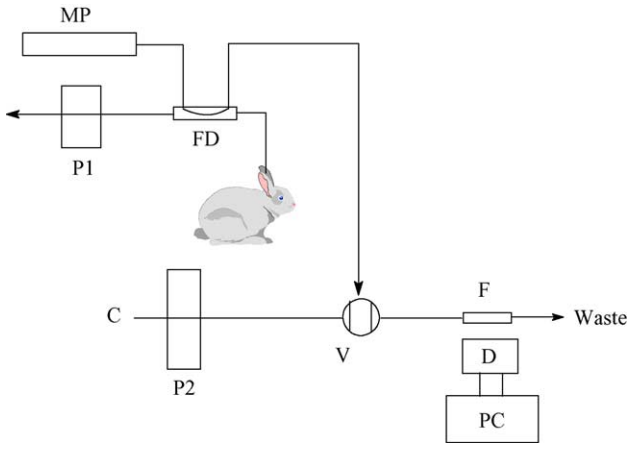

Fig. 2. Schematic diagram of flow system for in vivo on-line determination of ergometrine maleate in awake rabbit by microdialysis sampling. C: carrier flow (H_2O); MP: micropump and its controller system; FD: flow-through microdialysis probe; P1: variable-speed peristaltic pump (μl min^{-1}); P2: three channel peristaltic pump (ml min^{-1}); V: valve; F: flow cell; D: detector of F-4500 fluorescence spectrophotometer; PC: personal computer.

Fluorescence intensity was measured on the Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with a xenon-pulsed (10 s half-width, 50 Hz) excitation source. The excitation and emission slits were set at 2.5 and 5.0 nm, respectively. A conventional Perkin-Emer L2251247 flow-cell (25 μl volume) was used. A three channel peristaltic pump (Wenzhou, China) and an eight-channel injector valve (Wenzhou, China) were used to construct the FI system.

2.3. Fabrication of flow-through microdialyser

The preparation procedure of the flow-through microdialyzer was similar to that proposed by Fang et al. [15]. Two apertures (i.d. 0.6 mm) with a distance of 3.8 mm were made by needle through the wall of a micro-Line tubing (0.8 mm i.d., 1.2 mm o.d., 6.5 mm long, Thermoplastics, Chengdu Medical Appliance Corporation, China). A linear microdialysis probe (MF-7051) from Bioanalytical System (BAS) (West Lafayette, IN, USA) was then threaded into the micro-line tubing from one aperture and out from another aperture, and ensured that the dialysis membrane of probe completely be in the micro-line tubing. The connections between the linear microdialysis probe and the micro-line tubing were sealed with epoxy.

2.4. In vitro experiments

In these experiments, aqueous standard solutions were used instead of real samples from tested rabbit. The calibration of the measurement was performed by a flow-through microdialyzer in the Ringer's solution with variable concentrations of ergometrine maleate. The perfusion rate was used at 5 μl min^{-1}, and the sample flow rate was 15 μl min^{-1}. A different concentration of ergometrine maleate solution was

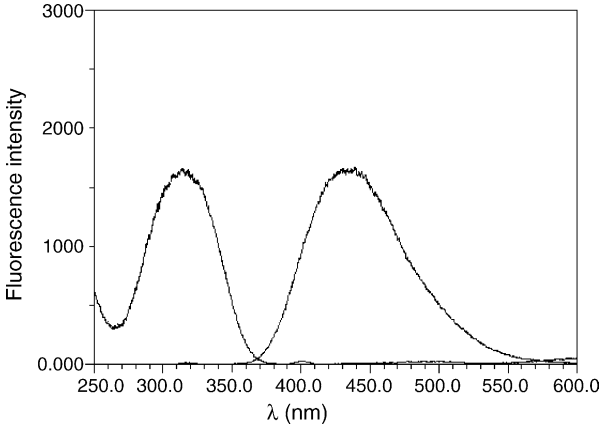

Fig. 3. Fluorescence excitation spectra (λ = 315.6 nm) and emission spectra (λ = 432.6 nm) of ergometrine maleate (0.1 μg ml^{-1}) in the Ringer's solution (148 mM NaCl, 4.0 mM KCl and 2.3 mM $CaCl_2$).

chosen randomly. After an equilibration time of 30 min, dialysis sample were collected every 3 min (170 s loading time and 10 s injection time). Perfusate (15 μl) in sample loop was injected into the carrier stream, and then a fluorescence signal was detected and recorded.

2.5. In vivo experiments

Experiments were carried out in female rabbits with body weight about 2.5 kg and age not less than 12 months. Movement of the test animal was limited by fixing it on a wooden dissecting plate (Huzhong Material Works, Henan, China). The body temperature of the rabbit was maintained at 37 °C with a heat pad. About 15 min before the experiments, the rabbit was given an intravenous injection of 5000 IU of heparin sodium injection via the edge of the test animal's ear to avoid clogging by blood coagulation during the experiments. A sterilized heparin-treated stainless-steel needle (0.6 i.d., 0.8 mm o.d., 20 mm long) was inserted into the edge vein of the test rabbit's ear. The outer end of the needle was connected to the flow-through microdialysis probe by a PTFE tubing (0.8 i.d., 2.0 o.d.), and the microdialysis sampling was performed by drawing out the blood of the test rabbit at a pump rate of 15 μl min^{-1}), while the perfusion rate was 5 μl min^{-1}. After 0.5 mg ergometrine maleate (0.2 mg kg^{-1}, i.v.) was administrated, further operations dealing for FI-fluorescence measurements were followed as described in the in vitro experiment section.

3. Results and discussion

3.1. The fluorescence of ergometrine maleate

Fig. 3 shows the excitation and emission spectra for Ringer's solution of ergometrine maleate. A maximum at 315.6 nm can be clearly observed in the excitation spectrum, the emission spectrum shows its maximum at 432.6 nm. These excitation and emission wavelengths were selected for the following assays to measure the fluorescence intensity.

3.2. Effect of sample flow rate on the fluorescence intensity

The effects of sample flow rate on analyte fluorescence intensity were studied for the flow-through microdialysis probe. The results indicate that the fluorescence intensity increased steeply with increasing of sample flow rates from 0 to 15 μl min^{-1}, above the sample flow rate of 15 μl min^{-1}, it remained almost constant. Then, 15 μl min^{-1} was the optimal sample rate for the system. This is in accordance with the result reported by Fang et al. [15]. Therefore, a flow rate of 15 μl min^{-1} was employed in in vivo experiments for achieving sufficient sensitivity without significantly disturbing the fluid balance of the test rabbit, even under extended periods of observation.

3.3. Effect of perfusion rate on the recovery

Various factors such as the perfusion rate, membrane length, temperature and type of analyte can affect the recovery of the microdialyzer. The perfusion rate is an important factor that defines the performance of a microdialyzer and has direct influence on the recovery of the microdialyzer. The effect of perfusion rate (from 2 to 10 μl min^{-1}) on ergometrine maleate recovery of the flow-through microdialyzer used in this study was investigated employing the MD FI-fluorescence system with the sample flow rate of 15 μl min^{-1}. The dialysate was loaded directly into the sample loop (about 40 μl) of the FI system. The loading time for each perfusion rate studied was long enough to ensure complete filling of the sample loop. The results are shown in Table 1. It can be seen that the recovery increases with decreasing the perfusion rate. A low rate of perfusion liquid through the microdialyzer gives a higher recovery, but a fewer injections could be made during a given time period and at high flow rates low concentration of ergometrine maleate reached the detection system. Considering the sensitivity and analysis efficiency, 5.0 μl min^{-1} was selected as the perfusion rate.

Table 1
Recovery of ergometrine maleate at different perfusion rate (sample flow rate: 15 μl min^{-1})

Perfusion rate (μl min^{-1})	Recovery (%)
2	54.7
3	41.5
5	32.2
7	11.2
10	7.6

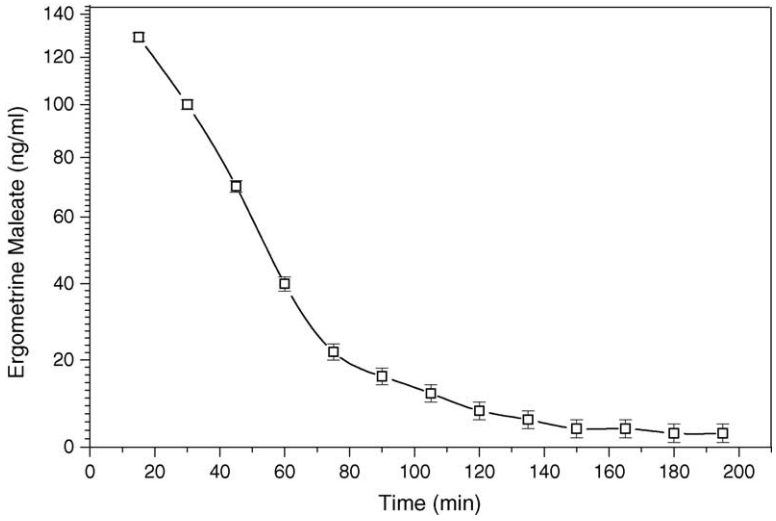

Fig. 4. The concentration of ergometrine maleate in test rabbit blood after administration (0.2 mg kg^{-1} bodyweight i.v., weight of the tested rabbit: 2.5 kg).

3.4. Performance of the on-line MD FI-fluorescence system

3.4.1. In vitro experiments

According to the method proposed by Fang et al. [15] that the sample loop was only partially filled in order to adapt the conventional injector with the lowest volume sample loop (15 μl) to couple with the microdialysis system, a perfusion rate of 5 μl min^{-1} and loading/injection time of 170/10 s were selected. Calibration graph of fluorescence intensity versus ergometrine maleate concentration was linear in the range of 1–140 ng ml^{-1}, and the detection limit was 0.3 ng ml^{-1} (3σ). The regression equation was $\Delta F = 51.5C + 10.4$ ($n = 9$, $r = 0.9989$, ΔF: the fluorescence intensity, C: the ergometrine maleate concentration in ng ml^{-1}).

3.4.2. In vivo experiments

Under the system in Fig. 2, the in vivo determination of ergometrine maleate in the blood of rabbit was carried out. The rabbit was administrated ergometrine maleate (0.2 mg kg^{-1}, i.v.), then the ergometrine maleate concentration in the blood was monitored by continuous sampling. Compensation for variation of sensitivity during in vivo monitoring was performed by the equation proposed by Fang et al. [15]:

$$C_x = \frac{kI_x I_{s_1}}{I_{s_2}}$$

where C_x is the ergometrine maleate concentration in sample x, k the slope of the calibration curve expressed in the concentration per unit peak height, I_x the peak height of sample x, and I_{s_1} and I_{s_2} are the peak heights of the sensitivity check standard at the beginning of the monitoring when the calibration curve was constructed and immediately after administrating sample x, respectively.

3.5. Pharmacokinetic studies

Fig. 4 shows the relationship of measured concentrations versus time for ergometrine maleate in rabbit blood after ergometrine maleate administration (0.2 mg kg^{-1}, i.v.). Based on these microdialysis sampling data, the pharmacokinetics of ergometrine maleate in the rabbit blood were performed utilizing the pharmacokinetic calculation software 'NDST-21' (China Pharmaceutical University, China). The results show that the concentration–time curve fitted to one-compartmental open model, and therefore the one-compartmental open model was chosen for calculation of pharmacokinetic parameters. Generally, the following equation can apply to a one-compartmental pharmacokinetic model [27]:

$$C = Ae^{-at}$$

where A is the concentration (C) intercept for fast or slow disposition phase, and a is the disposition rate constant for the disposition phase. Analysis of data after i.v. administration of ergometrine maleate (0.2 mg kg^{-1}) yielded the next

Table 2
Pharmacokinetic parameters of ergometrine maleate (0.2 mg kg^{-1} bodyweight i.v.) in rabbit blood

Parameters	Estimated
A (ng ml^{-1})	192
a (min^{-1})	0.0263
AUC (min ng ml^{-1})	5808
Cl (ml kg^{-1} min^{-1})	34.4
$T_{1/2}$ (min)	26.5

A: the concentration intercept for fast or slow disposition phase; a: the disposition rate constant; AUC: the area under the concentration–time curve; Cl: the body clearance; $T_{1/2}$: elimination half time.

equation:

$C = 192e^{-0.0263t}$

The pharmacokinetic parameters of ergometrine maleate are shown in Table 2.

4. Conclusion

The FI on-line microdialysis fluorescence system developed in this work was applied to monitor ergometrine maleate in rabbit blood. The concentration of ergometrine maleate in rabbit blood versus time was obtained after the administration (0.2 mg kg^{-1}, i.v.). The results indicate that the pharmacokinetics of ergometrine maleate in rabbit blood conform to a one-compartmental open model. Moreover this FI on-line fluorescence system can be used to other fluorescent analytes.

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