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Measurement and pharmacokinetic study of unbound tropisetron in rat blood by microdialysis with high-performance liquid chromatography

Chien-Tsai Huang [a], Chieh-Fu Chen [b], Tung-Hu Tsai [b,c,*]

[a] *Institute of Pharmacology, National Yang-Ming University, Taipei 112, Taiwan, ROC*
[b] *Department of Pharmacology, National Research Institute of Chinese Medicine, 155-1, Section 2, Li-Nong Street, Taipei 112, Taiwan, ROC*
[c] *Institute of Traditional Medicine, National Yang-Ming University, Taipei 112, Taiwan, ROC*

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Abstract

A microdialysis system coupled with liquid chromatography was applied for the measurement of unbound tropisetron in rat blood. The microdialysis probe was inserted into a jugular vein/right atrium of male Sprague–Dawley rats. Tropisetron (5 or 10 mg/kg, i.v.) was then administered via a femoral vein of rat. Samples were eluted with a mobile phase containing acetonitrile–100 mM monosodium phosphate (pH 5.0, 26:74, v/v). The UV detector wavelength was set at 284 nm. The detection limit of tropisetron was 20 ng/ml. The in vivo recovery of microdialysis probe in rat jugular vein/atrium for tropisetron at 1, 2, and 5 μg/ml were between 49 and 53% ($n = 5$). Intra- and inter-assay accuracy and precision of the analyses were $< 10\%$ in the range of 0.1–1 μg/ml. The method has been applied to pharmacokinetic analysis of unbound tropisetron in rat intravenous administration. Rapid distribution followed by a slower elimination phase was observed from the blood concentration–time curve. The disposition of tropisetron at each dose fitted well to a two-compartment model.

Keywords: Tropisetron; Microdialysis; Pharmacokinetics

1. Introduction

Tropisetron is a selective 5-HT_3 receptor antagonist which may have beneficial therapeutic effects in the treatment of vomiting and nausea resulting from cancer therapy (Lee et al., 1993;

* Corresponding author. Fax: +886-2-25328319.
E-mail address: thtsai@cma23.nricm.edu.tw (T.-H. Tsai)

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Falkson and Falkson, 1995). For the determination of tropisetron and its derivatives, a number of high-performance liquid chromatography techniques coupled with UV detection (Ofner et al., 1997; Brigas et al., 1998), fluorescence detection (Ofner et al., 1997) and mass spectrometric analysis (Sanwald et al., 1996) have been reported. However, most of these methods consume much time in sample clean-up programs. Currently, however, microdialysis provides a clean sampling method for concentration measurements without the need for clean-up procedures (Ungerstedt, 1984; Tsai et al., 1998).

Furthermore, there is another reason for choosing microdialysis. Microdialysis is an in vivo sampling technique that allows determination of drug concentration from protein unbound and extracellular space of most tissues (Hadwiger et al., 1994; Ofner et al., 1997). This is important because the total drug concentration (protein bound and unbound) in the blood does not reflect the concentrations at the cellular level, so that monitoring drug concentration in the interstitial space is crucial to understanding the time course of the antiemetic activity of tropisetron. In this study, we use microdialysis to provide near real-time analysis of tropisetron in blood dialysate samples after drug administration.

2. Experimental

2.1. Reagents

Tropisetron was purchased from Sandoz Pharma Ltd. (Basle, Switzerland). The sodium dihydrogen phosphate and reagents were obtained from Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Liquid chromatography

The liquid chromatographic system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an injector (Rheodyne 7125) and a UV detector (Some S-3702 ultraviolet detector, Tokyo, Japan). Analytes were separated using a reversed-phase SC18 column (Cosmosil, Kyoto, Japan; 150 × 4.6 mm I.D.; particle size 5 μm). Chromatography was performed at ambient temperature. The mobile phase containing acetonitrile–100 mM monosodium phosphate (pH 5.0) (26:74, v/v) with a flow rate of 1 ml/min. The mobile phase mixture was filtered through a 0.45 μm Millipore membrane, then, degassed prior to use. The UV wavelength was set at 284 nm. Output data from the detector were integrated via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.3. Animals

Adult, male Sprague–Dawley rats (280–320 g) were obtained from the Laboratory Animal Center at National Yang-Ming University (Taipei, Taiwan). These animals were specifically pathogen-free and allowed to acclimate in our environmentally controlled quarters (24 ± 1°C and 12:12 h light–dark cycle) for at least 5 days before being used for experiments. The rats were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and remained anesthetized throughout the experimental period. The rat's body temperature was maintained at 37°C with a heating blanket.

2.4. Microdialysis experiments

The blood microdialysis system consisted of a microinjection pump (CMA 100, Stockholm, Sweden) and a microcollector (CMA/140) for collection of dialysate sample (Huang et al., 1998). Blood dialysis probes were made of silica capillary and were of concentric designed dialysis membrane (Spectrum, 10-mm length, 150 μm outer diameter with a cut-off at nominal molecular weight of 13 000, Laguna Hills, CA, USA). The microdialysis probe (Fig. 1) was inserted into the jugular vein/right atrium and perfused with ACD solution (citric acid, 3.5 mM; sodium citrate, 7.5 mM; dextrose, 13.6 mM) at a flow rate of 2 μl/min using the CMA/100 microinjection pump. After obtaining 2 h base-line collection, tropisetron (5 or 10 mg/kg) was intravenously administered via a femoral vein. Dialysis samples were collected every 12 min and 24 μl of dialysate was

assayed with HPLC system (Tsai and Chen, 1996).

2.5. Recovery of microdialysis

A retrograde calibration technique was used for the study of in vivo recovery. The blood microdialysis probe was inserted into the rat jugular vein under anesthesia with sodium pentobarbital. ACD solution containing tropisetron (1, 2, or 5 μg/ml) was passed through the probe at a constant flow rate (2 μl/min) using the microinjection pump (CMA/100). A total of 2 h after the probe implantation, which was a stabilization period, the inlet (C_{in}) and outlet (C_{out}) concentrations of tropisetron were determined by HPLC. The in vivo recovery ratio was then calculated by the following equation (Sato et al., 1996):

$$\text{Recovery}_{\text{in vivo}} = 1 - (C_{out}/C_{in})$$

2.6. Method validation

All calibration curves of analyses were made prior to the experiments with correlation values of at least 0.995. The intra-day and inter-day variabilities for tropisetron were assayed (six replicates) at concentrations of 0.1, 0.2, 0.5 and 1 μg/ml on the same day and on 6 sequential days,

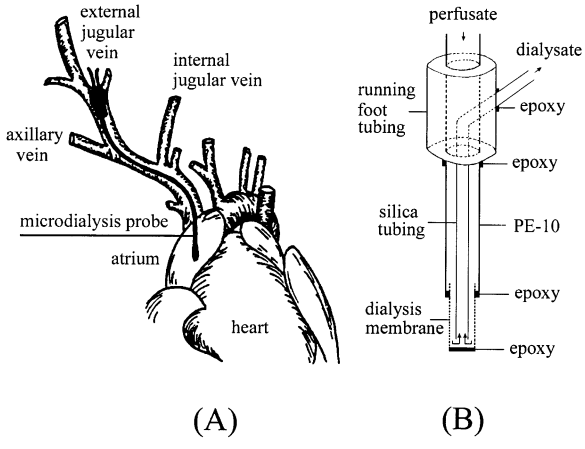

Fig. 1. The microdialysis probe was used in the rat blood. (A) a microdialysis probe inserted into the rat jugular vein/right atrium; (B) detailed description of a homemade microdialysis probe.

respectively. The accuracy (% bias) was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentration (C_{obs}) as follows:

$$\text{bias } (\%) = [(C_{nom} - C_{obs})/(C_{nom})] \times 100$$

The precision coefficient of variation (CV) was calculated from the observed concentrations as follows:

$$\%\ \text{CV} = [\text{standard deviation (S.D.)}/C_{obs}] \times 100$$

Accuracy (% bias) and precision (% CV) values of within $\pm 15\%$ were considered acceptable over this concentration range (Causon, 1997).

2.7. Pharmacokinetic analysis

Pharmacokinetic calculations were performed on each individual set of data.

Blood data were fitted to a biexponential decay given by the following formula:

$$C = A\ e^{-\alpha t} + B\ e^{-\beta t}$$

The distribution and elimination rate constants, α and β were calculated using the equation:

$$\alpha \text{ or } \beta = (\ln C_2 - \ln C_1)/(t_2 - t_1)$$

where C_1 is the value of C at time t_1 and C_2, the value of C at time t_2. Formation rate constants were calculated by extrapolation of the formation slope determined by the method of residuals. The areas under the concentration curves (AUCs) were calculated by the trapezoid method. Half-life ($t_{1/2}$) values were calculated using the equations:

$$t_{1/2,\alpha} = 0.693/\alpha \quad \text{and} \quad t_{1/2,\beta} = 0.693/\beta$$

for distribution and elimination half-life, respectively.

3. Results and discussion

Chromatograms for standard tropisetron, blank blood dialysate, and a typical chromatogram obtained from a rat sample after intravenous administration of tropisetron is shown in Fig. 2. Peak detection limit of tropisetron was 20 ng/ml at a signal-to-noise ratio of 3:1. No interfering peaks

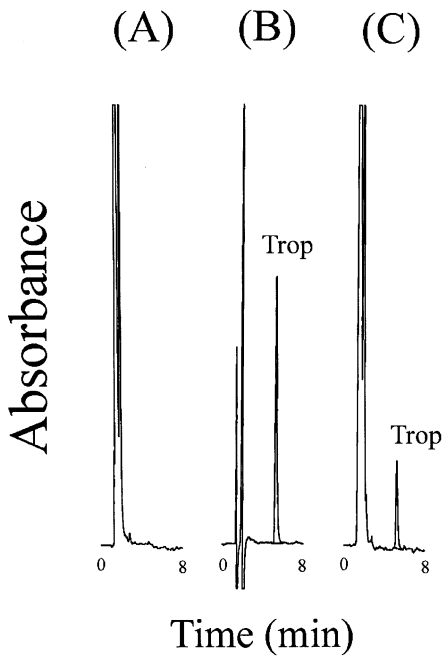

Fig. 2. Typical chromatogram of injection of (A) a blank blood dialysate; (B) standard tropisetron (1 μg/ml) and (C) a blood dialysate sample containing tropisetron (395.34 ng/ml) collected from jugular vein at 24 min after tropisetron administration (10 mg/kg, i.v.). Trop: tropisetron.

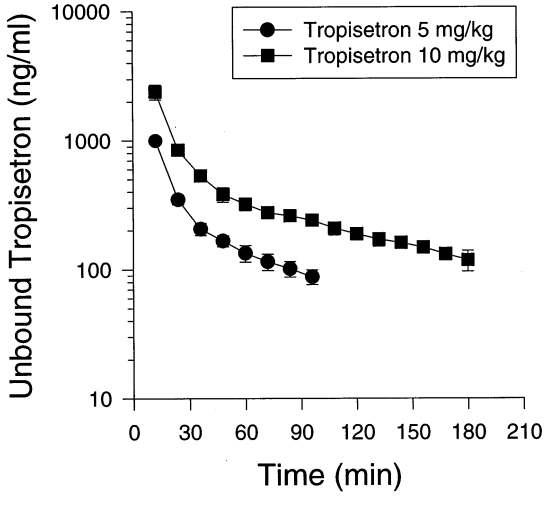

Fig. 3. Mean unbound levels of tropisetron in the rat blood after tropisetron administration (5 and 10 mg/kg, i.v.).

were observed within the time frame in which tropisetron was detected (Fig. 3A). The retention time of tropisetron was 5.3 min as shown in Fig. 3(B). These chromatographic conditions revealed no biological substances that would significantly interfere with the accurate determination of the drug, and we can therefore interpret the tropisetron peak on the chromatogram from the sample as accurately representing tropisetron. Fig. 3(C) depicts a chromatogram of actual tropisetron in rat blood.

The method was linear ($r^2 > 0.995$) over a concentration range 0.05–10 μg/ml for tropisetron. Intra-day and inter-day precision and accuracy for tropisetron (Table 1) fell well within predefined limits of acceptability. All % bias and % CV values were within $\pm 10\%$.

The in vivo recovery of microdialysis probe in rat jugular vein/atrium for tropisetron at concentration of 1, 2, and 5 μg/ml were calculated between 49 and 53% ($n = 5$) (Table 2).

The concentrations of tropisetron in dialysate of rat blood after tropisetron (5 or 10 mg/kg, i.v.) administration are shown in Fig. 3. The samples were collected at 12 min intervals during the entire experimental course. As described above, this method is applicable to further pharmacokinetic studies on tropisetron.

The distribution half-life ($t_{1/2,\alpha}$), elimination half-life ($t_{1/2,\beta}$) as well as distribution rate constant and elimination constant of tropisetron in rat blood are listed in Table 3. The dialysate samples collected over the first 2 h were discarded to allow recovery from the acute effects of the surgical procedure. The microdialysis sampling system and liquid chromatographic system were then applied to the pharmacokinetic characterization of tropisetron in rats.

This sampling method facilitates pharmacokinetic studies by reducing the effects of blood volume changes as compared with conventional blood withdrawing assays (De Lange et al., 1997). In addition, microdialysis is relatively inexpensive and easy to set up. Homemade probes further reduce expenses and allow customization for special needs such as sampling in blood.

Table 1
Intra-day and inter-day accuracy and precision of the HPLC method for the determination of tropisetron

Nominal concentration (ng/ml)	Observed concentration (ng/ml)*	CV (%)	Accuracy (% bias)
Intra-assay ($n=6$)			
100	102.98 ± 5.71	5.53	2.98
200	200.58 ± 6.61	3.29	0.29
500	494.42 ± 11.72	2.37	−1.12
1000	1001.51 ± 20.3	2.03	0.15
Inter-assay ($n=6$)			
100	101.22 ± 9.61	9.49	1.22
200	198.62 ± 5.09	2.56	−0.69
500	498.17 ± 7.56	1.52	−0.04
1000	999.64 ± 19.07	1.19	−0.04

* Observed concentration data are expressed as rounded means ± S.D.

4. Conclusion

A simple and sensitive liquid chromatographic system coupled to a microdialysis technique was developed for the determination of tropisetron the rat blood. Using the approach described in this report, the pharmacokinetics of unbound tropisetron in rat has investigated by microdialysis. Compared with other in vivo methods for pharmacokinetic study in the blood, microdialysis offers the advantages of being able to continuously monitor drug concentrations in the extracellular compartment in the same animals, causes less biological fluid loss and therefore minimal strain on hemodynamics. The results indicated that the disposition of protein free tropisetron in the blood appeared to fit a two-compartment model.

Table 2
In vivo microdialysis recovery (%) of tropisetron in rat blood[a]

Concentration (μg/ml)	Recovery (%)
1	49.03 ± 0.55
2	50.82 ± 0.79
5	52.36 ± 1.73

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

Table 3
Pharmacokinetic parameters of tropisetron in rat blood following drug administration (5 and 10 mg/kg, i.v.)[a]

Parameters	5 mg/kg	10 mg/kg
A, μg/ml	4.31 ± 0.61	12.71 ± 6.11
B, μg/ml	0.28 ± 0.013	0.54 ± 0.002
α, 1/min	0.14 ± 0.009	0.13 ± 0.029
β, 1/min	0.013 ± 0.0012	0.008 ± 0.0015
$t_{1/2,\alpha}$, min	4.89 ± 0.36	6.12 ± 1.41
$t_{1/2,\beta}$, min	55.93 ± 5.01	92.92 ± 17.21
AUC, μg/min/ml	52.93 ± 1.26	147.05 ± 26.49

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

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