ELSEVIER

International Journal of Pharmaceutics 241 (2002) 367–373

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Hepatobiliary excretion of fluconazole and its interaction with cyclosporin A in rat blood and bile using microdialysis

C.H. Lee [a], P.H. Yeh [a], T.H. Tsai [b,c,*]

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

Received 5 February 2002; received in revised form 1 April 2002; accepted 13 May 2002

Abstract

In order to investigate the hepatobiliary excretion of Fluconazole, we develop a rapid and sensitive method using high-performance liquid chromatography coupled with microdialysis for the simultaneous determination of unbound fluconazole in rat blood and bile. Microdialysis probes were inserted into both the jugular vein toward the right atrium and bile duct of male Sprague–Dawley rats for biological fluid sampling after administration of fluconazole at 10 mg/kg through the femoral vein. Fluconazole and dialysates were separated using a Zorbax phenyl column maintained at ambient temperature. The detection limit of fluconazole was 50 ng/ml. Biological fluid sampling thereby allowed the simultaneous determination of fluconazole levels in blood and bile. The disposition of fluconazole in the blood and bile fluid suggests that there was rapid exchange and equilibration between the blood and hepatobiliary system. In addition, to investigate the mechanism of *P*-glycoprotein related hepatobiliary excretion of fluconazole, we examined the drug–drug interaction of fluconazole and cyclosporin A in the aspect of pharmacokinetics. These results indicate that the plasma level of fluconazole was no different than that in bile, and that fluconazole undergoes hepatobiliary excretion, maybe unrelated to the *P*-glycoprotein transported system. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Fluconazole; Pharmacokinetics; Hepatobiliary excretion; Microdialysis; *P*-glycoprotein; Cyclosporin A

1. Introduction

Fluconazole is widely used for the treatment of systemic fungal infections and is considered as a first-line therapeutic choice for the treatment of vaginal candidiasis (Perry et al., 1995). However in some situations, due to the adverse effect of fluconazole, the concentration should be monitored. Although bioassay (Marchetti et al., 2001) and gas chromatographic (Rege et al., 1992) methods were originally used for the determination of fluconazole in body fluids, high-performance liquid chromatography (HPLC) (Koks et al., 1995; Cociglio et al., 1996) and capillary electrophoresis (von Heeren et al., 1996) methods were later developed to provide better accuracy

* Corresponding author. Tel.: +886-2-2820-1999x8091; fax: +886-2-2826-4276.
E-mail address: thtsai@cma23.nricm.edu.tw (T.H. Tsai).

0378-5173/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0378-5173(02)00272-7

and sensitivity. Method comparisons between bioassay and HPLC have been evaluated, showing good correlation, and both methods are useful for monitoring the serum level of fluconazole in routine clinical work (Cohen et al., 1997).

The LC-based approaches reported to date all result in the measurement of total drug concentration rather than the free fraction. Furthermore, some studies have attempted to monitor the protein-unbound form of fluconazole, focusing on the level in blood and brain (Yang et al., 1996, 1997). *P*-glycoprotein is expressed in normal tissues with excretory function such as liver, kidney, intestine, blood–brain barrier,…, etc. which is potentially resulted in drug–drug interaction. In order to reveal the role of *P*-glycoprotein involved in the hepatobiliary excretion of fluconazole, we measured protein-unbound fluconazole in rat blood and bile using microdialysis to study its hepatobiliary excretion and its interaction with cyclosporin A (a *P*-glycoprotein inhibitor). This method causes minimal disturbance to the hemodynamics and physiological processes of the experimental animal. The data thus obtained provide the basis for the construction of pharmacokinetic profiles and analyses.

2. Experimental

2.1. Chemicals and reagents

Fluconazole was purchased from Ortho-McNeil Pharmaceutical Inc. (Raritan, NJ). Cyclosporin A (Sandimmun) was obtained from Novartis Pharma (Basle, Switzerland). 1-octanesulfonic acid and orthophosphoric acid were purchased from Sigma Chemicals (St. Louis, MO), and E. Merck (Darmstadt, Germany), respectively. Chromatographic solvents were obtained from BDH (Poole, UK). Triple de-ionized water from Millipore (Bedford, MA) was utilized for all preparations.

2.2. Liquid chromatography

HPLC was performed with a chromatographic pump (BAS PM-80, West Lafayette, IN), a Rheodyne Model 7125 injector equipped with a 20 μl sampling loop and an ultraviolet detector (Linear Model 340, San Jose, CA). Separation was achieved by a reversed phase Zorbax extend C_{18} column (150×4.6 mm^2 I.D.; particle size 5 μm, Agilent, USA). The mobile phase consisted of methanol-1 mM 1-octanesulfonic acid (30:70, v/v, pH 3.0 adjusted with orthophosphoric acid), with a flow-rate of 1 ml/min, and the optimal wavelength was 210 nm. Output data from the detector were integrated using an EZChrom chromatographic data system (Scientific Software, San Roman, CA).

2.3. Assay validation

Quantitation was obtained by the measurement of drug concentration against the peak area of fluconazole. The concentration of unknown dialysate samples was determined by using the linear regression line (unweighted) of the concentration standard versus peak area. The precision of the method was expressed as the intra-day and inter-day coefficients of variation (%), which were assayed (six replicates) at concentrations of 0.1, 0.5, 1, and 5 μg/ml on the same day and on six sequential days, respectively. The accuracy (% Bias) was calculated from the nominal concentration (C_{nom}) and the mean value of the observed concentration (C_{obs}) as follows: Bias (%) $= [(C_{obs} - C_{nom})/(C_{nom})] \times 100$. The relative standard deviation (RSD) was calculated from the observed concentrations as follows: precision (% RSD) = (standard deviation (S.D.)/C_{obs}) $\times 100$. Accuracy and precision values within $\pm 15\%$ covering the actual range of experimental concentrations were considered acceptable (Causon, 1997).

2.4. Animals

The institutional animal experimentation committee of the National Research Institute of Chinese Medicine reviewed and approved all experimental protocols involving animals. Male, specific pathogen-free Sprague–Dawley rats were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei. The animals had free access to food (Laboratory ro-

dent diet # 5P14, PMI Feeds Inc., Richmond, IN) and water until 18 h prior to being supplied for experiments, at which time only food was removed. The rats were initially anaesthetized with urethane 0.8 g/ml and α-chloralose 0.08 g/ml (1 ml/kg, i.p.), and remained anaesthetized throughout the experimental period. The femoral vein was exposed for further drug administration. The rats' body temperature was maintained at 37 °C with a heating pad during the experiment.

2.5. Blood and bile microdialysis

Blood and bile microdialysis systems were comprised of a CMA/100 microinjection pump (CMA, Stockholm, Sweden) and microdialysis probes. The dialysis probes for blood (10 mm in length), and bile (7 cm in length) was made of silica capillary in a concentric design (Tsai et al., 1999, 2000; Tsai, 2001). Their tips were covered by dialysis membrane (Spectrum Lab., 200 μm inner diameter with a cut-off at nominal molecular weight of 13 000, Laguna Hills, CA) and all unions were cemented with epoxy. At least 24 h was allowed for the epoxy to dry. The blood microdialysis probe was located within the jugular vein/right atrium and then perfused with anticoagulant dextrose (ACD) solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) at a flow rate of 1.6 μl/min employing the microinjection pump (Tsai et al., 2000). The bile duct microdialysis probes were constructed in our own laboratory based on the design originally described by Scott and Lunte (1993) and Hadwiger et al. (1994). This bile microdialysis method was as reported in our previous studies (Tsai et al., 1999, 2000; Tsai, 2001).

A retrograde calibration technique was utilized during in vivo recovery. The blood, and bile microdialysis probes were inserted into the rats' jugular vein, and bile duct under anesthesia. Following a stabilization period of 2-h post probe implantation, the perfusate (C_{perf}) and dialysate (C_{dial}) concentrations of fluconazole were determined by HPLC. ACD solution (for blood microdialysis) containing fluconazole or Ringer's solution (for bile microdialysis) containing fluconazole was perfused through the probe at a constant flow rate (1.6 μl/min) employing the infusion pump (CMA/100). The in vivo relative recovery (R_{dial}) of fluconazole across the microdialysis probe was calculated by the following equation: $R_{dial} = (C_{perf} - C_{dial})/C_{perf}$. The microdialysate recovery and concentration calculations were performed according to our previous reports (Tsai, 2001; Tsai et al., 2001). Fluconazole microdialysate concentrations (C_m) were converted to unbound concentration (C_u) as follows: $C_u = C_m/R_{dial}$.

2.6. Drug administration

After a 2-h post-surgical stabilization period after probe implantation, the drug was subsequently administered according to the following study design. Six animals were used in each group. The control group received 10 mg/kg of fluconazole by i.v. bolus injection. For the cyclosporin A treated group, cyclosporin A 20 mg/kg was injected via the femoral vein 10 min before fluconazole 10 mg/kg injection. Outflow dialysates from blood, and bile were collected in a fraction collector (CMA/140) every 15 min. These dialysate samples were measured by HPLC during the same experimental day.

2.7. Pharmacokinetics

Pharmacokinetic calculations were performed on each individual animal's data utilizing the pharmacokinetic calculation software WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc, Apex, NC) by a noncompartmental method. The area under the concentration–time curve (AUC), the area under the first moment curve (AUMC) and the mean residence time (MRT) was calculated by statistical moments (Gabrielsson and Weiner, 1994). Formation rate constants were calculated from the extrapolated formation slope determined by the residual method. The AUCs from time zero to time infinity (AUC_{0-inf}) were calculated by the trapezoidal rule and extrapolated to time infinity by the addition of AUC_{t-inf}. An analogous method was employed to calculate the AUMC with the concentration vs. time data, as follows:

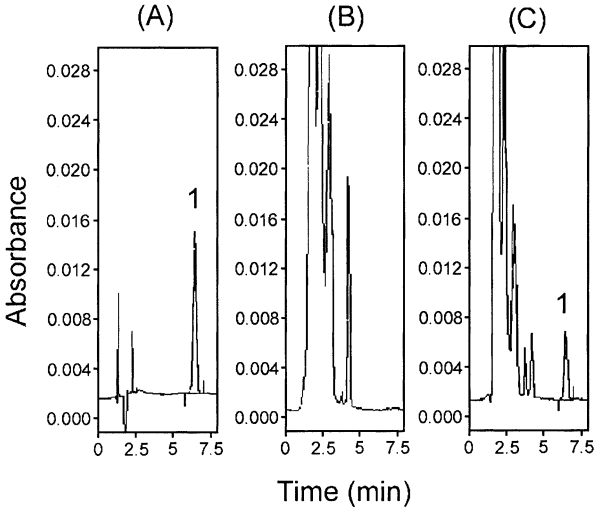

Fig. 1. Typical chromatograms of (A) standard fluconazole (5 μg/ml), (B) blank blood dialysate from the microdialysis probe before drug administration, and (C) blood dialysate sample containing fluconazole (2.1 μg/ml) collected from the rat blood microdialysate 240 min post fluconazole administration (10 mg/kg, i.v.). 1: fluconazole.

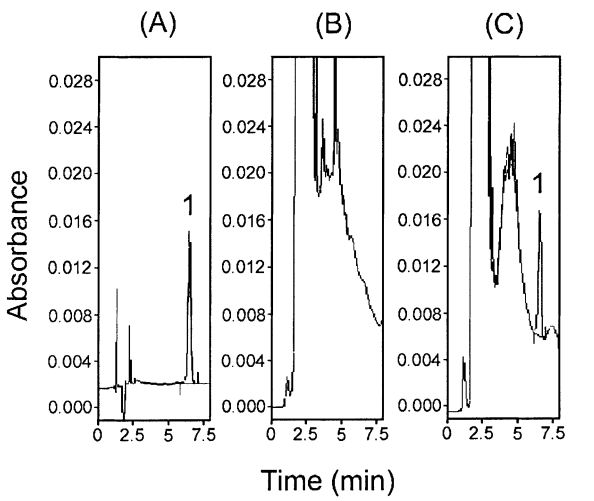

Fig. 2. Typical chromatograms of (A) standard fluconazole (5 μg/ml), (B) blank bile dialysate from the flow-through microdialysis probe before drug administration, and (C) bile dialysate sample containing fluconazole (4.3 μg/ml) collected from rat bile microdialysate 340 min after fluconazole administration (10 mg/kg, i.v.). 1: fluconazole.

$$\mathrm{AUC} = \mathrm{AUC}_{0-t} + \mathrm{AUC}_{t-\inf} = \mathrm{AUC}_{0-t} + C_{\mathrm{last}}/\lambda_z$$
$$\mathrm{AUMC} = \mathrm{AUMC}_{\mathrm{last}} + (t_{\mathrm{last}} C_{\mathrm{last}}/\lambda_z) + C_{\mathrm{last}}/(\lambda_z)^2$$

where C_{last} and t_{last} are the last observed concentration and time, respectively; and λ_z is the terminal slope which is estimated by linear regression of the logarithmic value of the last observed data. The clearance (Cl) and MRT were calculated as follows: $\mathrm{Cl} = \mathrm{dose}/\mathrm{AUC}$ and $\mathrm{MRT} = \mathrm{AUMC}/\mathrm{AUC}$. The blood to tissue distribution was calculated as follows: $\mathrm{AUC}_{\mathrm{tissue}}/\mathrm{AUC}_{\mathrm{blood}}$.

2.8. Statistical analysis

The statistical analysis was performed with SPSS version 10.0 (SPSS Inc., Chicago, IL). One-way ANOVA was followed by a Dunnett's post hoc test comparison between the control (fluconazole treated alone) and cyclosporin A treated groups. All statistical tests were performed at the two-sided 5% level of significance.

3. Results

Typical chromatograms of fluconazole in rat blood and bile are shown in Figs. 1 and 2, respectively. Separation of fluconazole from some endogenous chemicals in the blood, and bile dialysates were achieved in a mobile phase containing 30% methanol and 70% 1 mM 1-octanesulfonic acid at pH 3.0. Retention time of fluconazole was 6.5 min. The calibration curve of fluconazole was obtained prior to LC analysis of dialysates over concentration ranges of 0.1–10 μg/ml. Fig. 1A shows a standard injection of fluconazole (5 μg/ml), and Fig. 1B shows the chromatogram of a blank blood dialysate. None of the observed peaks interfered with the analyte. Fig. 1C shows the chromatogram of a blood dialysate sample containing fluconazole (2.1 μg/ml) collected from a rat blood microdialysis probe 240 min after fluconazole administration (10 mg/kg, i.v.).

Furthermore, none of the observed peaks interfered with the analyte in the chromatogram of the bile sample. Fig. 2A shows a standard injection of fluconazole (5 μg/ml). Fig. 2B shows a chromatogram of a blank bile dialysate sample ob-

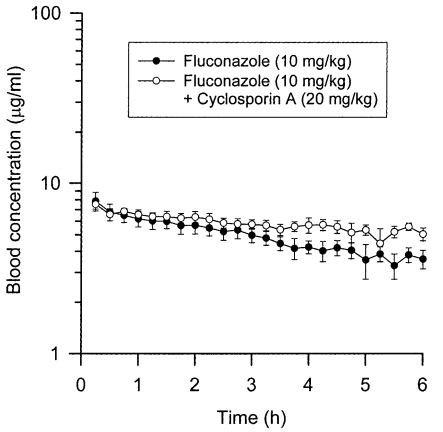

Fig. 3. Mean unbound levels of fluconazole in rat blood after fluconazole (10 mg/kg, i.v.) administration, and after co-administration of fluconazole (10 mg/kg, i.v.) and cyclosporin A (20 mg/kg, i.v.) ($n = 5$). Data are presented as mean ± s.e.mean.

tained from the bile duct microdialysis probe before the drug administration. Fig. 3C shows the chromatogram of a bile dialysate sample obtained fluconazole (4.3 μg/ml) collected from the bile duct microdialysis probe 340 min after fluconazole administration (10 mg/kg, i.v.).

This chromatographic system was validated for both intra- and inter-day accuracy (0.1–5.0%) and precision (0.1–5.1%) (Table 1). The chromatograms of blank blood and bile dialysate reveal that none of the observed peaks interfered with the analyte. Average in vivo recovery levels of fluconazole were 46.5 ± 2.4 in blood (1 and 5 μg/ml), and 81.6 ± 3.0% in bile (5 and 10 μg/ml) with $n = 6$ for each concentration (Table 2).

Table 2
In vivo microdialysate recovery (%) of fluconazole in rat blood and bile

Concentration (μg/ml)	Recovery (%)
Blood	
1	45.6 ± 3.0
5	47.4 ± 1.4
	Average: 46.5 ± 2.4
Bile	
5	81.4 ± 1.0
10	81.8 ± 2.6
	Average: 81.6 ± 2.9

Data are expressed as means ± S.D. ($n = 6$).

The concentration versus time curves of fluconazole in rat blood, and bile are shown in Figs. 3 and 4, respectively. The above pharmacokinetic curves reflect the fact that the disposition of fluconazole in rat bile exhibited a peak concentration after 30 min of fluconazole administration (10 mg/kg), followed by a slow elimination phase. The AUCs of fluconazole in blood, and bile were 77.6 ± 18.8, and 68.5 ± 5.4 min μg/ml, respectively (Table 3), indicating that fluconazole may be excreted from blood to bile.

Table 1
Intra-day and inter-day precision (% RSD) and accuracy (% Bias) of the HPLC method for the determination of fluconazole

Nominal concentration (μg/ml)	Observed concentration (μg/ml)	RSD (%)	Bias (%)
Intra-day			
0.10	0.095 ± 0.003	3.2	−5.0
0.50	0.49 ± 0.004	0.8	−2.0
1.00	1.02 ± 0.005	0.5	2.0
5.00	5.01 ± 0.014	0.3	0.2
10.00	9.99 ± 0.006	0.1	−0.1
Inter-day			
0.10	0.099 ± 0.005	5.1	−0.1
0.50	0.495 ± 0.025	5.1	−1.0
1.00	0.99 ± 0.04	4.0	−1.0
5.00	5.07 ± 0.09	1.8	1.4
10.00	9.96 ± 0.05	0.5	−0.4

Data are expressed as means ± S.D. ($n = 6$).

4. Discussion

The distribution ratio of fluconazole from blood to bile (AUC_{bile}/AUC_{blood}) in the control group was 0.88 ± 0.07. The lack of significantly different levels of AUC between the blood and bile agree with the previous report, which indicated that fluconazole levels in the bile were equal to those in the blood for the first 8 h (Bozzette et al., 1992). Another previous report suggests that a very high proportion of fluconazole is excreted unchanged in the urine, where concentrations of the drug are 10–20-fold higher than that in blood (Wildfeuer et al., 1997). Fluconazole is primarily excreted through the kidney.

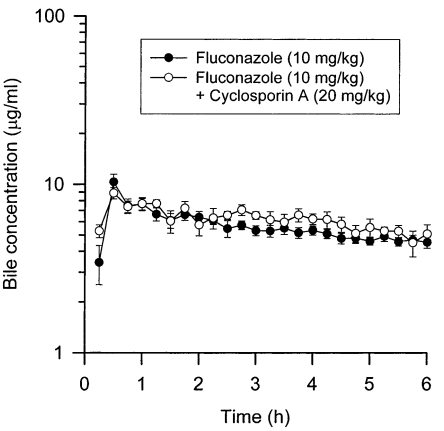

Fig. 4. Mean fluconazole in rat bile after fluconazole (10 mg/kg, i.v.) administration and co-administration of fluconazole (10 mg/kg, i.v.) and cyclosporin A (20 mg/kg, i.v.) ($n = 5$). Data are presented as mean ± s.e.mean.

Table 3
Pharmacokinetic parameters of the control group, with fluconazole administration (10 mg/kg, i.v.); and the treated group, with cyclosporin A 20 mg/kg injected via femoral vein 10 min prior to fluconazole 10 mg/kg injection

Parameters	Control group	Treated group
Blood		
AUC (min μg/ml)	77.6 ± 18.8	97.1 ± 20.4
MRT (min)	12.1 ± 3.7	12.3 ± 2.6
Bile		
AUC (min μg/ml)	68.5 ± 5.4	70.9 ± 16.0
MRT (min)	8.7 ± 0.8	$7.6 \pm .0$
AUC_{bile}/AUC_{blood}	0.88 ± 0.07	0.73 ± 0.16

Data are expressed as means ± s.e.mean ($n = 5$).

The multidrug resistance protein is overexpressed in several normal tissues and tumor cells. In the hepatocytes, drugs and their metabolites are excreted across the hepatocytes canalicular membrane into bile mediated by a primary active ATP-dependent conjugate export pump. These hepatobiliary excretion of xenobiotics are involve several transporter such as multispecific organic anion transporter (Oude Elferink and Jansen, 1994), non-bile acid organic anion transporter (Arias et al., 1993), glutathione *S*-conjugate export pump (Ishikawa, 1992), lukotriene export pump (Keppler, 1992), or *P*-glycoprotein transporter (Johnstone et al., 2000). Recently, an increasing number of cases of clinical resistance against fluconazole have been reported (Wirsching et al., 2000). To investigate the mechanisms of *P*-glycoprotein related hepatobiliary excretion of fluconazole, we focused on drug–drug interaction of fluconazole and cyclosporin A. Our results indicate that the distribution ratio of fluconazole from blood to bile (AUC_{bile}/AUC_{blood}) in the cyclosporin A treated group was 0.73 ± 0.16, was not significantly different from the control group (0.88 ± 0.07) (Table 3). Cyclosporin A (20 mg/kg) also did not markedly affect the biliary distribution ratio of fluconazole. These results imply that the hepatobiliary excretion of fluconazole might not be regulated by the *P*-glycoprotein.

The present study demonstrates that microdialysis allows extracellular concentration of drugs in blood and other biological tissues to be measured in vivo (Stahle, 1991). Furthermore, this sampling method facilitates pharmacokinetic study by reducing the influence of biological volume changes compared to conventional biological fluid withdrawing assays. In summary, a rapid and sensitive HPLC system for the determination of fluconazole in rat blood and bile were developed. This method exhibits no endogenous interference and the sensitivity is sufficient for the measurement of protein-unbound fluconazole in blood and bile. Fluconazole undergoes hepatobiliary excretion but might not be regulated to *P*-glycoprotein transported system.

Acknowledgements

This study was supported in part by research grants from the National Science Council (NSC90-2113-M-077-002; NSC90-2320-B-077-005), Taiwan.

References

Arias, I.M., Che, M., Gatmaitan, Z., Leveille, C., Nishida, T., St Pierre, M., 1993. The biology of the bile canaliculus. Hepatology 17, 318–329.

Bozzette, S.A., Gordon, R.L., Yen, A., Rinaldi, M., Ito, M.K., Fierer, J., 1992. Biliary concentrations of fluconazole in a patient with candidal cholecystitis: case report. Clin. Infect. Dis. 15, 701–703.

Causon, R., 1997. Validation of chromatographic methods in biomedical analysis. Viewpoint and discussion. J. Chromatogr. B 689, 175–180.

Cociglio, M., Brandissou, S., Alric, R., Bressolle, F., 1996. High-performance liquid chromatographic determination of fluconazole in plasma. J. Chromatogr. B 686, 11–17.

Cohen, L.G., DiBiasio, A., Lisco, S.J., Hurford, W.E., 1997. Fluconazole serum concentrations and pharmacokinetics in an obese patient. Pharmacotherapy 17, 1023–1026.

Gabrielsson, J., Weiner, D., 1994. Non-compartmental analysis. In: Pharmacokinetic and Pharmacodynamic Data Analysis Concepts and Applications. Swedish Pharm. Press, Stockholm, pp. 621–626.

Hadwiger, M.E., Telting-Diaz, M., Lunte, C.E., 1994. Liquid chromatographic determination of tacrine and its metabolites in rat bile microdialysates. J. Chromatogr. B 655, 235–241.

Ishikawa, T., 1992. The ATP-dependent glutathione *S*-conjugate export pump. Trend Biochem. Sci. 17, 463–468.

Johnstone, R.W., Ruefli, A.A., Smyth, M.J., 2000. Multiple physiological functions for multidrug transporter *P*-glycoprotein? Trend Biochem. Sci. 25, 1–6.

Keppler, D., 1992. Leukotrienes: biosynthesis, transporter, inactivation and analysis. Rev. Physiol. Biochem. Pharmacol. 121, 1–30.

Koks, C.H.W., Rosing, H., Meenhorst, P.L., Bult, A., Beijnen, J.H., 1995. High-performance liquid chromatographic determination of the antifungal drug fluconazole in plasma and saliva of human immunodeficiency virus-infected patients. J. Chromatogr. B 663, 345–351.

Marchetti, O., Majcherczyk, P.A., Glauser, M.P., Bille, J., Moreillon, P., Sanglard, D., 2001. Sensitive bioassay for determination of fluconazole concentrations in plasma using a Candida albicans mutant hypersusceptible to azoles. Antimicrob. Agents Chemother. 45, 696–700.

Oude Elferink, R.P.J., Jansen, P.L., 1994. The role of the canalicular multispecific organic anion transporter in the disposal of endo- and xenobiotics. Pharmacol. Ther. 64, 77–97.

Perry, C.M., Whittington, R., McTavish, D., 1995. Fluconazole. An update of its antimicrobial activity, pharmacokinetic properties and therapeutic use in vaginal candidiasis. Drugs 49, 984–1006.

Rege, A.B., Walker-Cador, J.Y., Clark, R.A., Lertora, J.J., Hyslop, N.E., George, W.J., 1992. Rapid and sensitive assay for fluconazole which uses gas chromatography with electron capture detection. Antimicrob. Agents Chemother. 36, 647–650.

Scott, D.O., Lunte, C.E., 1993. In vivo microdialysis sampling in the bile, blood and liver of rats to study the disposition of phenol. Pharm. Res. 10, 335–342.

Stahle, L., 1991. In: Robinson, T.E., Justice, J.B. Jr. (Eds.), Techniques in the Behavioral and Neural Science, vol. 7. Elsevier, Amsterdam, pp. 155–174.

Tsai, T.H., Huang, C.T., Shum, A.Y.C., Chen, C.F., 1999. Pharmacokinetic and biliary excretion study of esculetin by microdialysis in rat. Life Sci. 65, 1647–1655.

Tsai, T.H., Chen, Y.F., Chen, K.C., Shum, A.Y.C., Chen, C.F., 2000. Concurrent quantification and pharmacokinetic analysis of cefotaxime in rat blood and brain by microdialysis and microbore liquid chromatography. J. Chromatogr. B 738, 75–81.

Tsai, T.H., 2001. Pharmacokinetics of pefloxacin and its interaction with cyclosporin A, a *P*-glycoprotein modulator, in rat blood, brain and bile using simultaneous microdialysis. Brit. J. Pharmacol. 132, 1310–1316.

Tsai, T.H., Lee, C.H., Yeh, P.H., 2001. Effect of cyclosporin A, a *P*-glycoprotein modulator on the disposition and biliary excretion of camptothecin using microdialysis. Brit. J. Pharmacol. 134, 1245–1252.

von Heeren, F., Tanner, R., Theurillat, R., Thormann, W., 1996. Determination of fluconazole in human plasma by micellar electrokinetic capillary chromatography with detection at 190 nm. J. Chromatogr. A 745, 165–172.

Wildfeuer, A., Laufen, H., Schmalreck, A.F., Yeates, R.A., Zimmermann, T., 1997. Fluconazole: comparison of pharmacokinetics therapy and in vitro susceptibility. Mycoses 40, 259–265.

Wirsching, S., Michel, S., Morschhauser, J., 2000. Targeted gene disruption in Candida albicans wild-type strains: the role of the MDR1 gene in fluconazole resistance of clinical Candida albicans isolates. Mol. Microbiol. 36, 856–865.

Yang, H., Wang, Q., Elmquist, W.F., 1996. Fluconazole distribution to the brain: a crossover study in freely-moving rats using in vivo microdialysis. Pharm. Res. 13, 1570–1575.

Yang, H., Wang, Q., Elmquist, W.F., 1997. The design and validation of a novel intravenous microdialysis probe: application to fluconazole pharmacokinetics in the freely-moving rat model. Pharm. Res. 14, 1455–1460.