Quantitation of Talinolol in Rat Plasma By LC–MS–MS

Yan Li[1], Yoshiyuki Shirasaka[1,2], Peter Langguth[3], and **Ikumi Tamai[1,2]**

[1]Department of Membrane Transport and Pharmacokinetics, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamasaki, Noda, Chiba 278-8510, Japan; [2]Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan; and [3]School of Pharmacy, Johannes Gutenberg-University, Mainz, 55099, Germany

Abstract

The aim of this study was to develop and validate an assay based on liquid chromatography–tandem mass spectrometry to quantitate talinolol in rat plasma. After a simple protein precipitation step, separation was performed by reversed-phase liquid chromatography using gradient elution with acetonitrile–water–formic acid. Electrospray ionization in the positive ion mode with multiple reaction monitoring was used to analyze talinolol employing propranolol as internal standard. The calibration curve for talinolol was linear over the concentration range 1–250 ng/mL with a correlation coefficient > 0.995. The method was sensitive (limit of quantitation, 1 ng/mL) and had acceptable accuracy (85–115% of true values) and precision (intra- and inter-assay CV < 15%). Recovery of talinolol at concentrations of 1, 25, and 250 ng/mL was >78%. The method was successfully applied to the determination of the oral pharmacokinetics of talinolol in rat.

Introduction

P-gp is the product of the multidrug resistance gene (MDR1 in humans, mdr1a/1b in rats) (1,2) . It is an efflux transporter located on the plasma membranes of many cancer cells as well as in many normal tissues such as intestine, liver, kidney and brain (3). Various physicochemically and pharmacologically unrelated drugs have been shown to be P-gp substrates, including calcium channel blockers (e.g., verapamil), β-antagonists (e.g., talinolol), digitalis glycosides (e.g., digoxin), topoisomerase interactive agents (e.g., etoposide), immunosuppressive agents (e.g., cyclosporine A), and HIV-1 protease inhibitors (e.g., saquinavir, ritonavir, and nelfinavir) (4–11). Accumulating evidence suggests that P-gp significantly limits oral absorption of some drugs with low or moderate passive permeability (e.g., talinolol, digoxin), resulting in the low oral bioavailability of these drugs (5–9). Reversal of P-gp-based nonlinear oral pharmacokinetics may represent a clinical or formulation strategy for dose optimization. For example, co-administration of a P-gp inhibitor cyclosporine A enhanced the human bioavailability of paclitaxel and docetaxel by 7- and 10-fold, respectively, and reduced interpatient variability in the systemic exposure of docetaxel to that seen with IV administration (7,8).

*Author to whom correspondence should be addressed: email tamai@p.kanazawa-u.ac.jp.

P-gp also acts in concert with CYP3A4 to decrease systemic exposure of their common substrates (12–14). There is growing clinical evidence showing that the oral bioavailability of many bisubstrates of CYP3A and P-gp can be increased by concomitant administration of CYP3A and P-gp inhibitors (15). Thus, it is necessary to distinguish the contribution of intestinal P-gp by using a non-metabolized P-gp probe such as talinolol (16,17). Indeed, the oral bioavailability of talinolol in human can be increased or decreased significantly when coadministered with the P-gp inhibitor erythromycin or pretreated with P-gp inducing herb St. John's Root, respectively (18,19). In addition, grapefruit juice significantly increased talinolol bioavailability in rat possibly due to similarly inhibitory effects on P-gp while the precise mechanism remains unclear (20).

To support clinical pharmacokinetic studies, various assays have been developed to determine plasma or serum talinolol concentrations that typically employ an aliquot of 0.2–1 mL plasma or serum samples (21–23). These methods can often fulfill the requirement of quantification of talinolol in clinical trials. However, due to the limited volume of rat blood, these assays cannot be applied in determining oral talinolol pharmacokinetics in rats. Thus, a sensitive and specific assay for talinolol in rat plasma is required to support talinolol pharmacokinetic studies in rat. In this study, we report the development and validation of an liquid chromatography–tandem mass spectrometry (LC–MS–MS) method for the quantitation of talinolol in rat plasma. This method has also been applied into the oral talinolol pharmacokinetic studies in rats.

Experimental

Chemicals and reagents

Talinolol racemate (purity 98.5%) was kindly provided by the Arzneimittelwerk Dresden (AWD, Radebeul, Germany). Naringin were purchased from Chromadex (Irvine, CA). Methanol and acetonitrile (LC–MS-grade) were purchased from Wako (Tokyo, Japan). Milli-Q water, prepared from demineralized water, was used throughout the study (Millipore, Billerica, MA). Other reagents were analytical-grade and used without further purification.

LC–MS–MS parameters

The LC–MS system consisted of an API 3200 Triple Quadrupole mass spectrometer (MDS-Sciex, Concord, ON, Canada) equipped with an Shimadzu LC-20AD HPLC binary

pump and autosampler (Kyoto, Japan). Electrospray ionization in the positive ion mode was employed. To optimize MS–MS conditions, talinolol and propranolol (internal standard, IS) (Figure 1) standard solutions (50 ng/mL in 50% acetonitrile–water) were simultaneously infused into ion source using a syringe pump at 10 µL/min. Multiple reaction monitoring (MRM) was used to detect the transitions at *m/z* 364.3 → 100.2 for talinolol and *m/z* 260.2 → 116.1 for IS. Mass spectrometer parameters were: source spray voltage 3.5 kV; ion source temperature 450°C; declustering potential 46 V; collision energy 33 V for talinolol and 27 V for IS; collision cell exit potential 4.0 V. Nitrogen was used as curtain and collision gas. Analyst version 1.4 was used for data manipulation.

Chromatographic conditions

Separation of talinolol and IS was achieved using a Capcell Pack C_{18} analytical column (10 cm × 4.6 mm, i.d., 5 µm) (Shiseido, Tokyo, Japan) protected by Mightysil C_{18} precolumn (20 mm × 4.6 mm i.d., 5 µm) (Kanto Chemical, Tokyo, Japan). The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B) delivered as a linear gradient as follows: 0–2.0 min, 30–95% B; 2.0–2.5 min, 95% B; 2.5–2.6 min, 95–30% B; 2.6–4.0 min, 30% B. The flow rate of the mobile phase is 0.6 mL/min. An in-line motorized six-port divert valve was used to divert the eluent flow to waste for the first 2 min and into mass spectrometer for 2–4 min.

Sample preparation

An aliquot (40 µL) of plasma sample was mixed with IS solution (40 µL, 80 ng/mL in acetonitrile) and 120 µL ice-cold acetonitrile. After vortexing for 20 s, the mixture was centrifuged at 12,000 × *g* for 10 min. The supernatant (120 µL) was collected and dried in a Speedvac (Savant Instruments, Farmingdale, NY) at room temperature. The residue was reconstituted in 60% acetonitrile–40% water (60 µL), and 10 µL was injected into the LC–MS system.

Assay validation

A talinolol stock solution (1.0 mg/mL) in water was used to make a series of standard solutions in acetonitrile. Spiked standards (1–250 ng/mL) in rat plasma were used to prepare standard curves based on peak area ratios of talinolol to IS. Linearity was assessed by weighted ($1/x^2$) linear least-squares regression analysis. The limit of quantitation (LOQ) was defined as the minimum concentration which could be determined with acceptable accuracy (i.e. mean relative error < 20%) and precision [coefficient of variation (CV) < 20%]. Accuracy and precision were determined by analysis of low, medium, and high quality control (QC) samples (1, 25, and 250 ng/mL, respectively) prepared by spiking rat plasma with independently prepared standard solutions. Matrix effects were evaluated by comparing LC–MS–MS chromatograms after injections of water, talinolol in water, extracted blank plasma, and spiked rat plasma. Stability was assessed by analyzing low, medium, and high QC samples on storage at room temperature (20°C) for 3 h and at –30°C for 14 days. Stability of reconstituted samples on storage in autosampler vials at 4°C for 12 h was also assessed.

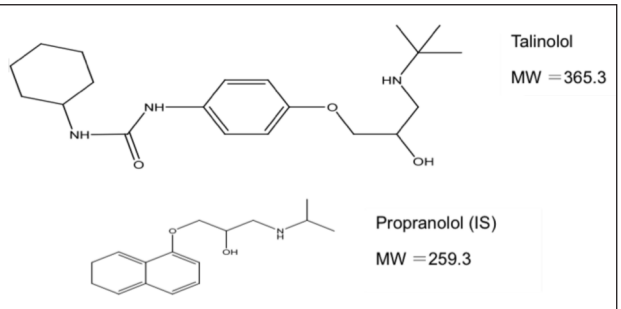

Figure 1. Chemical structures of talinolol and propranolol.

Pharmacokinetic study

Experiments were approved by Tokyo University of Science Animal Ethics Committee. Adult male Wistar rats (220 ± 20 g body weight) were provided by Tokyo University of Science. The animals were free of specific pathogen and were allowed to adapt to their environmentally controlled conditions (24 ± 1°C and 12:12 h light-dark cycle). All rats were cannulated in the right jugular vein, allowed to recover, and administered a single dose of talinolol dissolved in water for injection. Groups of rats (n = 4–5) were administered an oral dose of talinolol solution (10

Table I. Accuracy and Precision of the LC–MS–MS Method for the Analysis of Talinolol in Rat Plasma (n = 5)

	Precision		Accuracy
Nominal conc. (ng/mL)	Measured conc. (ng/mL) (mean ± SD)	CV (%)	Mean relative error (%)
Intra-assay			
1	0.92 ± 0.06	7.32	–8.27
25	22.86 ± 2.83	12.38	–8.56
250	235.80 ± 6.11	2.81	–5.68
Inter-assay			
1	1.03 ± 0.12	11.8	2.8
25	24.93 ± 1.33	5.35	–0.3
250	222.75 ± 4.92	2.21	–10.9

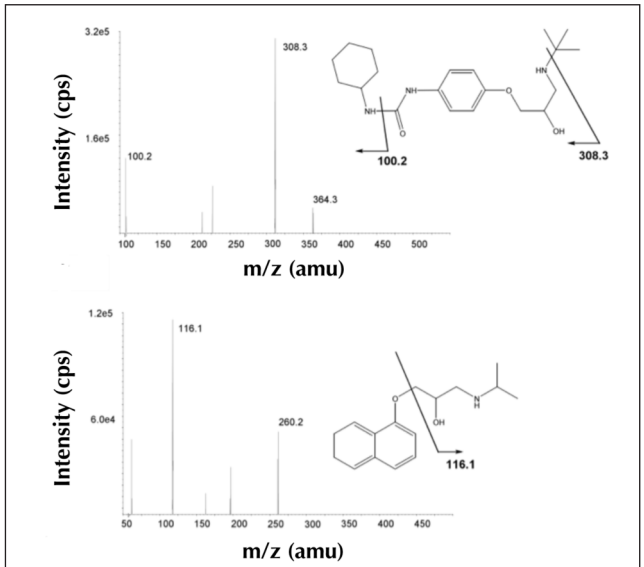

Figure 2. MS–MS spectra of (A) talinolol 33 V collision energy and (B) IS (propranolol) 27 V collision energy.

mg/kg, 2 mg/mL, pH 7.0) in the presence or absence of naringin (20 and 200 µM) by gavage. Blood samples (250 µL) were collected from the cannula into heparinized tubes for up to 24 h following dosing. Each blood sample was replaced by an equal volume of saline and heparinized saline was used to maintain catheter patency. Blood samples were centrifuged at 3,000 $g \times 10$ min, and plasma samples were collected and stored at –30ºC until analysis.

Plasma concentrations of talinolol were analyzed using a non-compartmental method based on statistical moment theory. Area under the plasma concentration-time curve from 0 to 6 h (AUC_{0-6}) was calculated by using of the linear trapezoidal rule. The maximum plasma drug concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were obtained directly from the experimental data. The apparent elimination half-life of log-linear phase was calculated based on the terminal elimination rate constant determined by the log-linear regression of the final data points (at least 3).

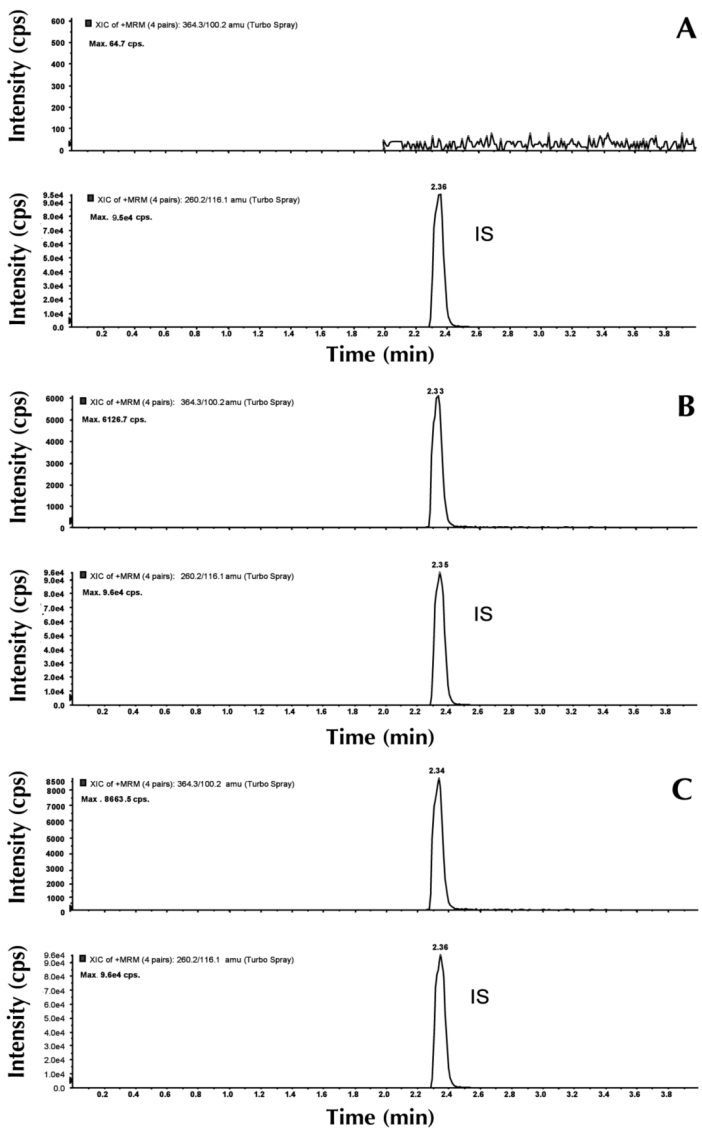

Figure 3. Representative MRM chromatograms of talinolol and IS in rat plasma: (A) blank plasma spiked with IS, (B) plasma sample at the LOQ, and (C) plasma sample from a rat 6 h after oral dose of 10 mg/kg.

Results and Discussion

Method development

Talinolol (pKa 9.4) is present as a cation at acidic pH ranges, and ESI in the positive ion mode was chosen as the ionization source in the present study. Full-scan mass spectral analysis of talinolol and IS showed molecular ions at *m/z* 364.3 and 260.2, respectively. The MS–MS product ion spectrum of the molecular ion (*m/z* 364.3) and the predominant fragmentation patterns are shown in Figure 2A. The most abundant ions in the product-ion mass spectra of talinolol were at 308.0 and 100.2 while for IS the most abundant ion in the product-ion mass spectra is 116.1 (Figure 2A–2B). However, the loss of 56 Da (C_4H_8) may not be selective for analysis of talinolol in biological samples (22). Thus the transitions *m/z* 364.3 → 100.2 and *m/z* 260.2 → 116.1 were used for MRM monitoring of talinolol and IS, respectively. The dwell times were both set at 150 ms for talinolol and IS. Declustering potential and collision cell exit potential were optimized to values of 46 V and 4 V, respectively. The collision energy was set at 33 V and 27 V for the analyte and IS, respectively. It was found that an ion source temperature < 350°C gave a poor response and the maximum intensity of product ions was obtained at 450°C. A decrease in the source spray voltage from 5.0 kV to 3.5 kV caused a significant increase in fragmentation and sensitivity.

A single-step protein precipitation using ice-cold acetonitrile was employed to facilitate speed and convenience. The absolute recovery for talinolol at three QC concentrations was > 78%, indicating low matrix effects for the analyte. For talinolol separation, three types of mobile phase A (e.g. 0.1% formic acid, 0.1% acetic acid, and 10 mM ammonium acetate in Milli Q water) were tested. Formic acid was finally chosen because it produces a better signal-noise ratio and peak shape for both the analyte and IS.

Assay validation

Representative chromatograms of talinolol in rat plasma are shown in Figure 3. Matrix-specific interfering peaks that required modification of the mobile phase composition were not observed (Figure 3). There is no significant change of the absolute values of talinolol and IS peak areas in the presence of naringin 2 mM in plasma samples at three QC concentrations ($n = 3$). The standard curve for talinolol in rat plasma was linear over the concentration range 1–250 ng/mL with a correlation coefficient > 0.995 and an insignificant non-zero intercept ($n = 5$). Precision and accuracy were < 15% at all QC concentrations as shown in Table I. The LOQ corresponded to the lowest standard (1 ng/mL) on the standard curve, which allowed the method to be applied to a pharmacokinetic study in rat. The stability of talinolol under various storage conditions is summarized in Table II.

Pharmacokinetic study

Plasma concentration-time curves for talinolol are shown in Figure 4. After oral administration, talinolol was rapidly absorbed and gave a maximum plasma concentra-

tion (C_{max}) of 157 ± 24 ng/ml at 1–1.5 h after oral administration. Thereafter, talinolol concentration declined and can be quantified until 6 h after administration. In the presence of naringin 200 µM, C_{max} and $AUC_{0-6\,h}$ increased 81% and 93%, respectively, and there is no change of T_{max} and the elimination half-life (Table III), suggesting that naringin may significantly enhance the oral absorption of talinolol due to inhibition of rat intestinal P-gp. Indeed, it has been reported that naringin significantly inhibits P-gp-mediated talinolol transport across Caco-2 cell monolayers (24). In current study, the naringin concentration is far less than its concentration in grapefruit juice (1–2 mM) (24), which implies that naringin is a major inhibitor of rat intestinal P-gp in grapefruit juice. Further studies by using rat P-gp-expressing cells may be required to elucidate the mechanisms of interaction between P-gp and naringin.

Table II. Stability of Talinolol in Rat Plasma Under Various Conditions (*n* = 3)

Storage condition	Nominal conc. (ng/mL)	Measured conc. (ng/mL) (mean ± SD)	Mean relative error (%)
20°C/3 h	1	0.92 ± 0.10	–8.50
	25	22.55 ± 3.17	–9.8
	250	237.25 ± 22.91	–5.1
4°C/12 h	1	1.04 ± 0.09	3.50
Reconstituted samples	25	25.73 ± 1.19	2.93
	250	231.50 ± 21.83	–7.40
–30°C/14 days	1	1.01 ± 0.13	1.30
	25	25.70 ± 1.62	2.80
	250	220.67 ± 3.21	–11.73

Table III. Pharmacokinetic Parameters of Oral Talinolol (10 mg/kg) With or Without Co-administeration of Naringin 20 or 200 µM*

	Control	+ Naringin (20 µM)	+ Naringin (200 µM)
$AUC_{0-6\,h}$ (h·ng/mL)	175 ± 22	193 ± 32	338 ± 65*
C_{max} (ng/mL)	157 ± 24	154 ± 12	284 ± 55*
t_{max} (h)	1.3 ± 0.3	1.7 ± 0.3	1.0 ± 0.1
Elimination half life (h)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1

* Data are means ± S.E.M.; *n* = 4.

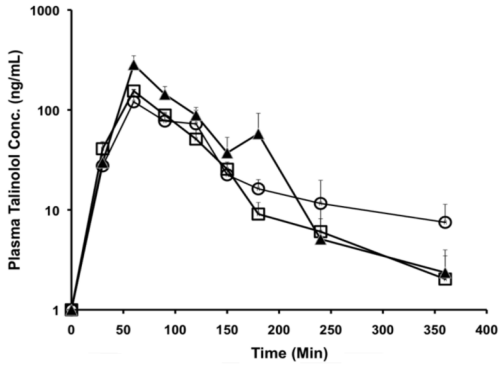

Figure 4. Concentration-time curves for talinolol for 6 h after an oral dose in rat in the presence of naringin (20 µM, blank squares; 200 µM, solid triangles) or absence of it (control, blank circles). Data are means + S.E.M., *n* = 4.

Conclusion

In conclusion, a rapid and sensitive LC–MS–MS method for the determination of talinolol in rat plasma has been developed and validated. The method is suitable for pharmacokinetic studies and has been applied in a naringin-talinolol pharmacokinetic interaction study in rat.

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