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Study of the effect of Wuzhi tablet (*Schisandra sphenanthera* extract) on tacrolimus tissue distribution in rat by liquid chromatography tandem mass spectrometry method

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ABSTRACT: A liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed and validated for determining tacrolimus (FK506) in rat tissues to study the effect of *Schisandra sphenanthera* extract on FK506 tissue distribution. After a liquid–liquid extraction with ethyl acetate, FK506 and ascomycin (IS) were subjected to LC-MS/MS analysis using positive electrospray ionization under multiple reactions monitoring mode. Chromatographic separation of FK506 and ascomycin was achieved on a Hypersil BDS C_{18} column with a mobile phase consisting of methanol-water (containing 2 mM ammonium acetate, 95 : 5, v/v). The intra- and inter-batch precision of the method were less than 8.8 and 9.8%, respectively. The intra- and inter-batch accuracies ranged from 97.5 to 104.0%. The lowest limit of quantification for FK506 was 0.5 ng/mL. The method was applied to a FK506 tissue distribution study with or without a dose of Wuzhi (WZ) tablet. Most of the FK506 tissue concentrations were slightly increased after a concomitant WZ tablet dose, but the whole blood concentration of FK506 was dramatically increased 3-fold after a concomitant WZ tablet dose. These results indicated that the LC-MS/MS method was rapid and sensitive enough to quantify FK506 in different rat tissues, and strict drug monitoring is recommended when co-administering WZ tablet in clinical use. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: tacrolimus (FK506); tissue distribution; liquid chromatography/tandem mass spectrometry; drug–drug interaction; *Schisandra sphenanthera* extract

Introduction

Tacrolimus (FK506, Prograf®), a macrolide lactone, is a well-known potent immunosuppressant for the prevention and/or treatment of graft rejection in solid organ transplantation patients for nearly two decades (Staatz and Tett, 2004; Mentzer *et al*., 1998; Bowman and Brennan, 2008). With its narrow therapeutic index, therapeutic drug monitoring is standard clinical practice in the management of transplant recipients (Venkataramanan *et al*., 1995; Jusko *et al*., 1995). It is known that the metabolism of tacrolimus occurs in the liver and the small intestine via the cytochrome P450 (mainly CYP3A4 and 3A5), and its absorption is also be limited due to the involvement of efflux transporter – P-glycoprotein (P-gp) (Iwasaki, 2007; Jeong and Chiou, 2006). Therefore, drug or compounds that inhibit or induce the CYP3A/P-gp may increase or decrease tacrolimus blood levels, respectively (van Gelder, 2002). Wuzhi (WZ) tablet is a preparation of ethanolic extract of Wuweizi (*Schisandra sphenanthera*), which contains 7.5 mg schisantherin A per tablet. Its major active chemical constituents include schisandrin A, schisandrin B, schisandrol A, schisandrol B, schisantherin A, schisantherin B and gomisin C (Huyke *et al*., 2007). WZ tablet is a prescribed drug [registration no. in China: WS-10557(ZD-0557)-2002] rather than a herbal supplement in clinical practice to improve liver dysfunction in chronic hepatitis patients (Loo *et al*., 2007).

Recently, it has been reported in Chinese healthy human volunteers that concomitant administration of WZ capsule [another preparation of *Schisandra sphenanthera* extract (SchE)] could enhance the *in vivo* whole blood concentration of FK506, which might be due to inhibition of CYP3A4 and/or P-gp via substances in SchE (Xin *et al*., 2007). Our group has observed similar phenomena in renal transplant patients as well as rats with about 2–3-fold increase in C_{max} and AUC after concomitant administra-

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Abbreviations used: SchE, *Schisandra sphenanthera* extract; WZ, Wuzhi tablet.

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tion of WZ tablet (data not shown). However, to our knowledge, the effect of WZ tablet on FK506 tissue distribution has not yet been clarified, and a sensitive and specific analytical method is required to determine FK506 in rat tissues in order to study the effect of WZ tablet on tacrolimus tissue distribution.

Various analytical methods have been reported for the measurement of FK506 in bio-matrix such as bioassays, enzyme immunoassays, radioreceptor monitoring, high-performance liquid chromatography (HPLC), and liquid chromatography tandem mass spectrometry (LC-MS/MS) (Kobayashi *et al*., 1991; Christians *et al*., 1991; Staatz *et al*., 2002; Napoli, 2006; Westley *et al*., 2007; Yuan *et al*., 2008; Li *et al*., 2008). However, most of the current analytical methods are for measurement of blood FK506 concentration in pharmacokinetic studies or therapeutic drug monitoring, and there are few methods available for the quantification of FK506 in tissues. Enzyme immunoassay was reported to measure the tissue FK506 concentration in mice (Yokogawa *et al*., 1999; Chiou *et al*., 2000). However, immunoassays have often been reported to have more positive deviation than the parent drug-specific LC-MS/MS method due to the nonspecific binding of the antibody to drug metabolites (Napoli, 2006; Westley *et al*., 2007). During the past few years, LC-MS/MS has become an increasingly important tool for FK506 measurement due to its high specificity and sensitivity compared to other available technologies including immunoassays (Westley *et al*., 2007). Until now, there has been no published report using LC-MS/MS method for determination of FK506 in rat tissues. Therefore, a more sensitive, reliable and rapid analytical method is required to determine FK506 in rat tissues in order to study the effect of WZ tablet on FK506 tissue distribution.

Take the above into consideration, the purpose of the current study was to develop a rapid and sensitive LC-MS/MS method to measure FK506 in rat tissue and apply the method to study the effect of WZ tablet on FK506 tissue distribution.

Experimental

Chemical and Reagents

FK506 with a purity of 98% as determined by HPLC with ultraviolet (UV) detection was synthesized and provided by Toronto Research Chemicals Inc. (Toronto, Canada). Ascomycin (FK520, as internal standard) with a purity of 95% as determined by HPLC with UV detection was synthesized and provided by BIOMOL Research Laboratories Inc. Ascomycin is a structural analog of FK506 with similar molecular weight and hydrophobicity and thus it was used as an internal standard (Plymouth Meeting, PA, USA). Prograf® capsules (1 mg of FK506 per capsule) and FK506 injection (5 mg × 1 mL) were produced by Astellas Ireland Co. Ltd. (Ireland), WZ tablets (each tablet containing 7.5 mg schisantherin A) were produced by Fanglue Pharmaceutical Company (Guangxi, China). Methanol and acetonitrile of HPLC grade were purchased from Tedia Company Inc. (Beijing, China). All other reagents were of analytical grade or HPLC grade when appropriate. Ultra-pure water was obtained from a Milli Q-plus system (Billerica, MA, USA).

Preparation of Standard and Quality Control Samples

The stock standard solutions of FK506 and FK520 were prepared by dissolving the accurately weighed individual compounds in methanol–water (50:50, v/v) to give a final concentration of 500 and 100 μg/mL, respectively. The solutions were then serially diluted with methanol–water (50:50, v/v) to obtain working solutions at concentrations over 0.05–4.0 μg/mL for FK506 and a working solution of FK520 at 2.0 μg/mL. The stock solutions of the analyte or IS were stored at −80°C, and the working solutions were stored at 4°C and were brought to room temperature before use.

The analytical standard and quality control (QC) samples were prepared by spiking blank rat tissue homogenates with standard working solutions during validation and each experimental run for tissue distribution study. Calibration samples were made at concentrations of 0.5, 2, 10, 50, 100, 200 and 400 ng/mL for FK506. Quality control samples were at concentrations of 2, 50 and 200 ng/mL.

Sample Preparation

Samples were prepared using procedure described by our previous report with slight modifications (Li *et al*., 2008). Briefly, to 100 μL of rat tissue homogenate in a 2 mL test tube, 10 μL of FK520 was added and vortexed for 30 s. Zinc sulfate solution (0.1 M, 400 μL) was added and mixed well to lyse the cells; this was followed by adding 400 μL acetonitrile and vortex-mixing for 1 min and standing at room temperature for 10 min. After centrifugation at 2500***g*** for 5 min, the supernatant was transferred to a clean 5 mL centrifuge tube and extracted with 1.5 mL extraction solvent ethyl acetate. After vortex-mixing for 1 min and standing at room temperature for 10 min, the mixtures were centrifuged at 2500***g*** for 10 min. The organic phase was then transferred to a clean 1.5 mL centrifuge tube and evaporated to dryness. The residues were dissolved in 100 μL mobile phase and an aliquot (10 μL) of the reconstituent was injected onto the LC-MS/MS for analysis.

Liquid Chromatographic and Mass Spectrometric Conditions

A Waters Alliance 2695 separation module (Avondale, CA, USA) was used for solvent and sample delivery. Chromatographic separation was achieved by using a C_{18} column (Hypersil BDS C_{18}, 3 μm particle size, i.d. 2.1 × 50 mm, Elite HPLC Inc., Dalian, China) at room temperature. The mobile phase consisted of methanol-water (95:5, v/v, containing 2 mM ammonium acetate in water), pumped at a flow rate of 200 μL/min. The total running time was 2 min for each sample.

A Quattro micro™ triple quadrupole mass spectrometer (Micromass, Notre Dame, IN, USA) equipped with an ESI source was used for mass analysis and detection. Mass spectrometric analysis was performed in the positive-ion mode (ESI^+) and set up in the multiple reaction monitoring (MRM) mode. Nitrogen was used as desolvation (550 L/h) and nebulizer gas (50 L/h). Argon was used as collision gas (0.0033 mbar). The capillary voltage was 3.0 kV for the analytes and the entrance and exit energies of the collision cell were set at 1 and 2 V, respectively. The source and desolvation temperatures were kept at 110 and 350°C, respectively. The cone voltage was 20 V for all analytes. The collision behavior was carried out using 25 eV collision energy for FK506 and IS, respectively. On the basis of the full-scan mass spectra of each analyte, the most abundant ions were selected and the mass spectrometer was set to monitor the transitions of the precursors to the product ions as follows: m/z 821.7 → 768.9 for FK506, and m/z 809.8 → 757.0 for IS. The dwell time for each analyte was set to 0.8 s. Data acquisition, peak integration, and calibration were performed with Massllynx V 4.0 software (Micromass).

Tissue Distribution Study

Fifteen male Sprague–Dawley rats weighing between 180 and 250 g were supplied by the Laboratory Animal Service Center, Sun Yat-sen University. The animals were kept in a 22–24°C room with a light/dark cycle of 12:12 h and 55–60% relative humidity. They had free access to standard rodent food and water. The rats were fasted for 12 h before the tissue distribution study. All studies were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China.

The rats were randomly divided into two groups with five rats in each group to receive oral administration of FK506. In group 1, rats orally

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received FK506 by gavage at a dose of 3.78 mg/kg/day. In group 2, 0.25 g/kg/day of SchE was given to rats orally 5 min before oral administration of 0.945 mg/kg/day FK506. All groups were dosed for 4 days and rats were sacrificed at 12 h post-dosing at day 4. A blood sample was removed immediately from the rat by cardiac puncture to collect the whole blood. Major organs and tissues such as the heart, liver, spleen, lung, kidney, brain, fat, muscle, testes, small intestine and thymus were removed. The tissues were quickly excised, rinsed well with ice-cold saline, blotted dry and weighed. The samples were homogenized in ice-cold saline to prepare 0.5 g/mL homogenates. Blank tissue homogenates were prepared in a similar manner using rats without prior exposure to FK506 or WZ tablet. The tissue homogenates were stored at −20°C until assay.

Results and Discussion

Method Development

ESI was chosen as the ionization source in this study. Consistent with our previous study (Li *et al*., 2008), FK506 and IS had low abundance of protonated molecular ions due to relatively weak proton affinity. Therefore, ammonium acetate was selected to add in the mobile phase to form ammonium-adduct ions, which had a peak (*m/z*) at (M_r + 18) and easily fragmented during MS/MS detection. The maximum sensitivity of ammonium-adduct ions was achieved when 2 mM ammonium acetate was added. Capillary and cone voltages and collision energies were optimized to obtain the greatest intensity of the most abundant product ion for further MS/MS experiments. Therefore, the multiple reaction monitoring (MRM) transitions of *m/z* 821.7 → 768.9 for FK506 and *m/z* 809.8 → 757.0 for IS were selected to obtain maximum sensitivity. Positive-ion ESI-MS/MS product-ion spectrum of these two compounds are shown in Fig. 1.

A liquid–liquid extraction (LLE) procedure with ethyl acetate was used to extract FK506, diltiazem and IS from human whole blood in our previous report (Li *et al*., 2008). A similar LLE procedure with slight modifications was used in this study to extract FK506 and IS from rat tissues. The extraction efficiencies from human whole blood and rat tissues were compared during our method development. In representative rat tissues such as liver and kidney, the extraction efficiency of FK506 ranged over 41.7–47.8%, slightly lower than that of human whole blood (with a range of 58.3–62.6%). However, the extraction recoveries at all analyte concentrations were similar without concentration dependence (Table 3). Thus, similar LLE procedure was still used to extract the analytes from rat tissues throughout the study.

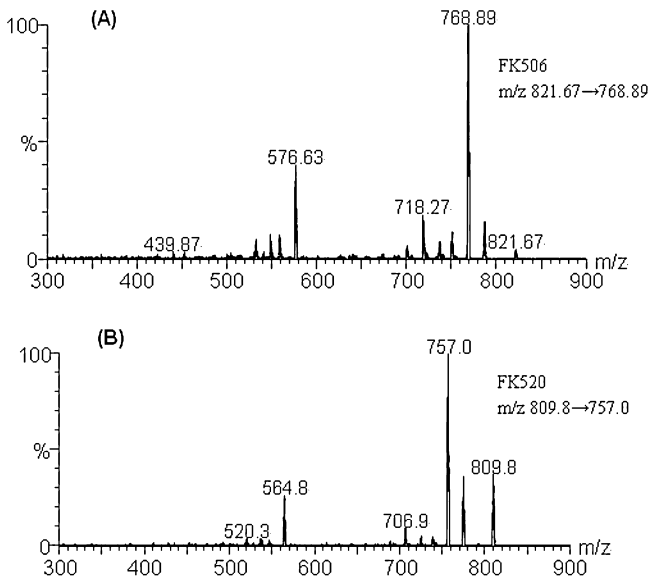

Figure 1. Representative full-scan product ion spectra of the ammonium-adduct ion molecules of FK506 (A) and ascomycin (FK520, B).

Method Validation

Selectivity and Matrix Effects

The selectivity towards endogenous matrix was tested in six different batches of rat tissue samples by analyzing blanks and samples at the LLOQ. There was no significant interference at the expected retention times of the analytes and the IS. Representative MRM chromatograms of the analytes in rat liver and kidney are showed in Fig. 2. The retention times for FK506 and IS were 1.20 and 1.19 min, respectively. The method had a short run time (2.0 min) for determination of FK506 and ascomycin.

Since potential matrix effect is a concern with the fast isocratic system, the co-elution effect and potential ion suppression were evaluated. Absolute and relative matrix effect (ME) on the spectral response of the analyte and IS was assessed using the procedure described by Matuszewski *et al*. (2003) and Bi *et al*. (2006). Matrix effect data at different QC concentrations in five different lots of rat tissues (liver and kidney) are presented in Table 1. The absolute ME values were 97.6–109.7% indicating no significant ion suppression or enhancement effect. The variability was acceptable with RSD values <9.9% at different concentrations. These data confirm that the relative matrix effect for the analyte was not significant. Thus, no ion suppression or enhancement effect was observed and the present analytical method was considered reliable.

Linearity and Lower Limit of Quantification

Linearity was assessed by analyzing FK506 samples over 0.5–400 and 0.5–300 ng/mL concentration ranges in rat tissues and blood. The slope, the intercept, and the correlation coefficient (*r*) for each standard curve from each analytical run were determined automatically by Massllynx version 4.0 software program. Table 2 shows the representative regression equations of the standard curve, correlation coefficient values and linear ranges for FK506 in rat tissues and blood. The squared correlation coefficients (r^2) for the daily calibration curves were all >0.99. Overall, FK506 showed satisfactory linearity over the studied concentration ranges in rat tissues.

The lowest concentration on the calibration curve of FK506 was 0.5 ng/mL. The analytes' response at this concentration level in all bio-matrix were >5 times the baseline noise. The precision and accuracy at this concentration level in all bio-matrix were acceptable, with ≤10.8% of the CVs and range between −2.5 and 4.2% of the relative errors. Thus, the lowest concentration on the calibration curve was accepted as the LLOQ.

Precision and Accuracy

The intra-batch and inter-batch precision and accuracy data for FK506 in representative rat tissues are summarized in Table 3. All values of accuracy and precision were within recommended limits. Intra-batch precision ranged between 0.8 and 8.8%, and the inter-batch precision was between 1.6 and 9.8%. The mean intra-batch relative error was between −2.5 and 4.2%, and the mean inter-batch relative error was between −1.8 and 2.2%.

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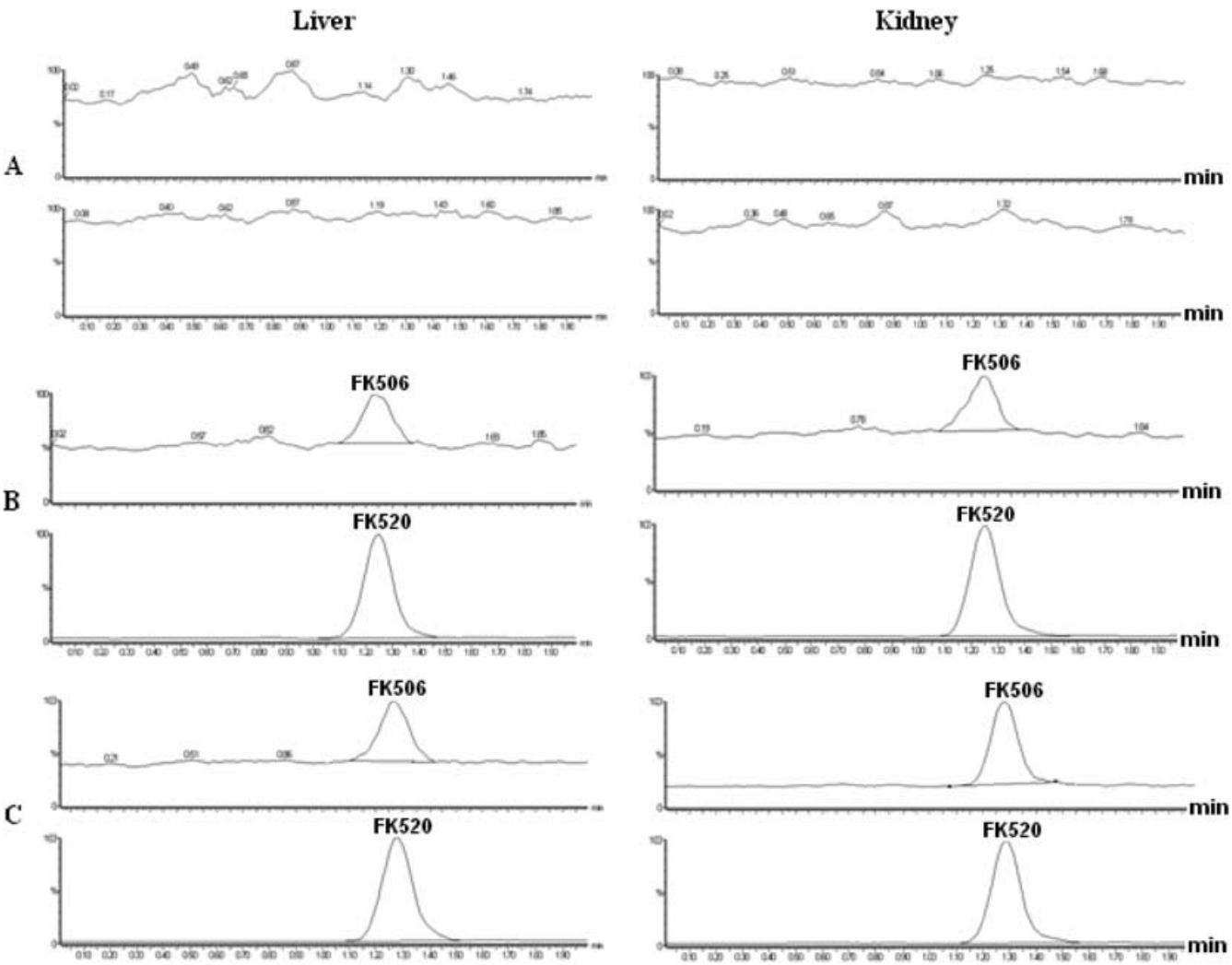

Figure 2. Representative MRM chromatograms of FK506 and ascomycin (FK520) in rat liver and kidney. (A) blank tissue sample; (B) blank tissue sample spiked with 0.5 ng/mL of FK506; (C) tissue samples from a rat after receiving multiple oral dose of FK506 and WZ tablet.

Table 1. Matrix effect data in five different lots of representative rat tissues ($n = 5$)

Compound/tissues	Nominal concentration (ng/mL)	ME[a] (mean ± SD, %)	RSD (%)
FK506 in liver	2.0	104.6 ± 6.6	6.3
	50.0	97.6 ± 3.0	3.0
	200.0	98.8 ± 5.7	5.8
FK506 in kidneys	2.0	109.7 ± 10.8	9.9
	50.0	107.3 ± 3.3	3.1
	200.0	98.6 ± 4.3	4.4

[a] The corresponding peak areas of the analyte spiked in tissue homogenates post-extraction (*B*) were compared with those of the standard solution at equivalent concentration (*A*). The ratio ($B/A \cdot 100$) is defined as the matrix effect (ME).

Recovery

Table 4 shows the recovery (extraction efficiency) of FK506 and IS from rat tissues following LLE procedure. The recovery of FK506 from rat liver and kidney homogenates ranged over 41.7–46.8 and 42.5–47.8%, respectively, and were similar at all analyte concentrations without concentration dependence. The recovery of IS was 42.9–44.0% from rat liver and kidney homogenates. These results indicated that the extraction efficiency for the analyte and IS was acceptable.

Tissue Distribution Study

To our knowledge, this is the first report to study the effect of WZ tablet (SchE) on the FK506 tissue distribution in rats. The tissue distribution study was conducted with oral doses of 3.78 mg/kg/

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Table 2. Standard curves, correlation coefficients and linear ranges of FK506 in tissue samples

Tissues	Regression equation of the standard curves	Correlation coefficients	Linear ranges (ng/mL)
Heart	$y = 0.010087x + 0.0092119$	0.9994	0.5–400
Liver	$y = 0.010389x + 0.0070224$	0.9990	0.5–400
Spleen	$y = 0.0093341x + 0.011191$	0.9998	0.5–400
Lung	$y = 0.010609x + 0.0057651$	0.9994	0.5–400
Kidneys	$y = 0.0099176x + 0.011397$	0.9988	0.5–400
Brain	$y = 0.0092259x + 0.044396$	0.9972	0.5–400
Fat	$y = 0.0095910x + 0.0072850$	0.9988	0.5–400
Testes	$y = 0.0098744x + 0.044486$	0.9989	0.5–400
Muscle	$y = 0.010001x + 0.0064654$	0.9999	0.5–400
Small intestine	$y = 0.0096702x + 0.0071970$	0.9993	0.5–400
Thymus	$y = 0.010299x + 0.0094461$	0.9998	0.5–400
Blood	$y = 0.020784x + 0.0093149$	0.9993	0.5–300

Table 3. Intra- and inter-batch precision and accuracy data for assays of FK506 in representative rat tissues ($n = 5$)

Tissues	Nominal concentration (ng/mL)	Precision		Accuracy
		Mean ± SD	RSD[a] (%)	Mean relative error (%)
Intra-batch				
Liver	2.0	2.06 ± 0.18	8.8	2.75
	50.0	50.81 ± 1.85	3.6	1.62
	200.0	195.00 ± 2.02	1.0	−2.50
Kidneys	2.0	2.08 ± 0.08	3.8	4.20
	50.0	50.04 ± 0.39	0.8	0.08
	200.0	197.60 ± 6.34	3.2	−1.20
Inter-batch				
Liver	2.0	1.97 ± 0.19	9.8	−1.50
	50.0	50.89 ± 1.63	3.2	1.78
	200.0	196.41 ± 3.11	1.6	−1.80
Kidneys	2.0	2.04 ± 0.10	5.1	2.20
	50.0	49.26 ± 1.41	2.9	−1.48
	200.0	196.38 ± 5.64	2.9	−1.81

[a] RSD = Relative standard deviation.

Table 4. The recovery (extraction efficiency) for FK506 and ascomycin in representative rat tissues ($n = 5$)

Compound/tissues	Nominal concentration (ng/mL)	Recovery[a] (mean ± SD, %)	RSD[b] (%)
FK506 in liver	2.0	41.7 ± 2.9	6.9
	50.0	44.5 ± 3.3	7.3
	200.0	46.8 ± 0.8	1.7
FK506 in kidneys	2.0	47.8 ± 0.8	1.6
	50.0	42.5 ± 2.7	6.3
	200.0	45.0 ± 3.1	6.8
Ascomycin in liver	200.0	42.9 ± 3.8	8.9
Ascomycin in kidneys	200.0	44.0 ± 2.0	4.6

[a] The recovery (extraction efficiency) of analytes from rat tissues after the extraction procedure was determined by comparing the areas of extracted analytes with that of the standard solutions that represent 100% recovery.
[b] RSD = relative standard deviation.

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day FK506-alone or 3.78 mg/kg/day FK506 with 0.25 g/kg/day SchE, and the rats were sacrificed at 12 h post-dose at day 4. The dose of FK506 and SchE to rats was converted from the clinical dose regimen to transplantation patients in clinical practice. The results of our preliminary pharmacokinetic interaction study in rats show that area under the blood concentration–time curve (AUC) of FK506 was increased 3–4-fold after a concomitant oral dose of 0.25 g/kg SchE to rats (data not shown). Therefore, in the group with SchE, the dose of FK506 was reduced to 0.945 mg/kg/day (one-quarter of the dose of FK506-alone group). From the preliminary pharmacokinetic study in rats, the half-life times of FK506 were 7.9 ± 0.7 and 8.7 ± 4.2 h for the FK506-alone group and the group with the extract. Usually, steady-state blood concentration will be reached after dosing for seven half-life times. Therefore, the dosing regimen lasted for 4 days and rats were sacrificed during steady state.

Table 5. Concentration of FK506 in tissues and blood after a concomitant oral dose of WZ tablet (*Schisandra sphenanthera* extract) to rats for 4 days ($n = 5$)

Tissues	Concentration (mean ± SD, ng/mL)	
	FK506-alone	FK506 + WZ tablet
Heart	13.77 ± 3.67	21.47 ± 8.68
Liver	1.33 ± 1.64	1.53 ± 0.76
Spleen	10.10 ± 1.61	14.70 ± 2.14*
Lung	20.96 ± 4.88	21.99 ± 4.15
Kidneys	7.92 ± 1.77	8.72 ± 1.32
Brain	2.97 ± 1.00	4.63 ± 0.42
Fat	7.96 ± 0.14	9.45 ± 0.83
Testes	5.58 ± 0.82	7.14 ± 1.98
Muscle	10.96 ± 1.10	15.23 ± 1.91*
Small intestine	18.96 ± 1.69	24.14 ± 3.76
Thymus	15.60 ± 2.46	17.02 ± 3.40
Blood	0.65 ± 0.39	1.72 ± 0.68*

* $p < 0.05$.

The concentrations of FK506 in rat tissues and blood after oral administration of FK506 with or without concomitant dose of SchE are listed in Table 5 and Fig. 3. The FK506 concentration in thymus was also assayed because the thymus FK506 concentration is closely related to its immunosuppressive effects. These results show that FK506 is widely distributed to the rat tissues after oral administration. The lung, small intestine, heart and thymus had higher FK506 concentration among all the detected tissues. Therefore, the tissue concentrations in the FK506-alone group were found to decrease in the order of lung > small intestine > thymus > heart > muscle > spleen > fat > kidneys > testes > brain > liver, and tissue concentrations in the group with the extract decreased in a similar order of small intestine > lung > heart > thymus > muscle > spleen > fat > kidneys > testes > brain > liver. The tissue concentration of FK506 in normal mice was reported in a previous study in the following order: lung > spleen > kidneys > heart > gut > brain > liver (Yokogawa *et al.*, 1999). The tissue concentration in the group with the extract was slightly higher than that of the FK506-alone group, but most of these differences were not significant. The blood FK506 concentration in the group with the extract was significantly higher than that of the FK506-alone group even when the dose of FK506 was significantly decreased. FK506 concentration in the brain and liver was lowest among all the detected tissues; this may be because FK506 is prevented from entering the brain by the blood–brain barrier and extensive metabolism occurred in the liver with high liver extraction. All together, these results suggest that SchE can significantly increase the FK506 whole blood concentration with slight change in FK506 tissue distribution. Thus, frequent and strict therapeutic drug monitoring of blood FK506 concentration and dose adjustment are highly recommended when coadministering SchE in clinical use.

Conclusions

A rapid and sensitive LC-MS/MS method was developed to determine FK506 concentrations. The method was validated according to FDA (2001) guidance and showed high sensitivity, reliability,

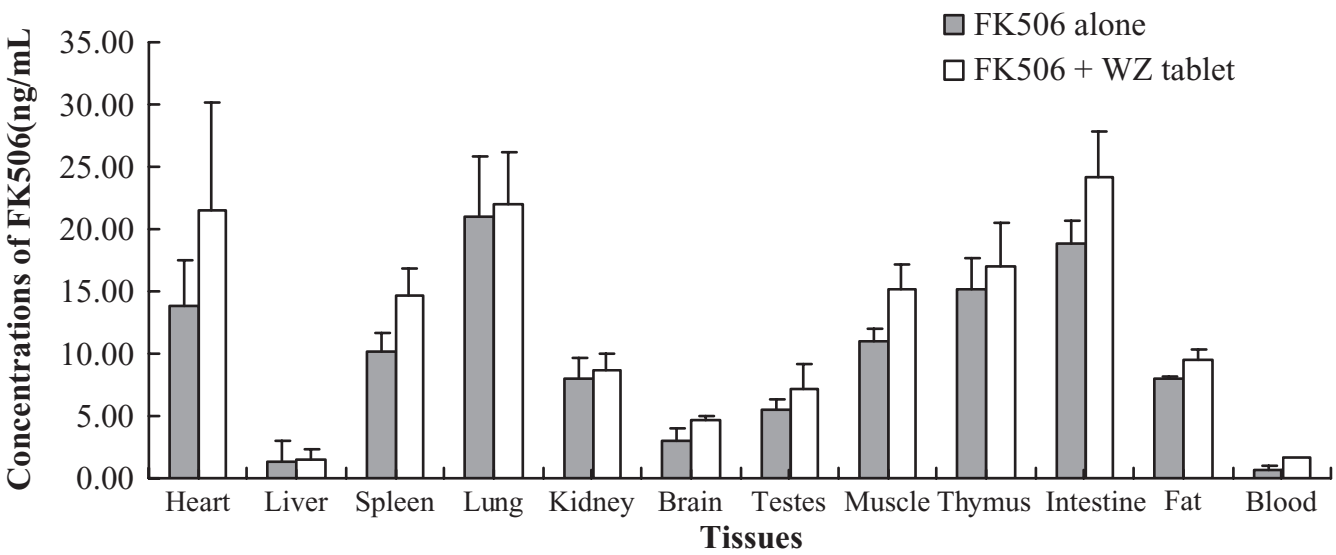

Figure 3. Concentration of FK506 in the tissues and blood of rats after receiving multiple oral dose of FK506 with or without concomitant dose of WZ tablet ($n = 5$).

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specificity and excellent efficiency with a total run time of 2.0 min per sample. The method was successfully applied for the first time in studying the effect of WZ tablet on the FK506 tissue distribution in rats. The results of tissue distribution study indicated that a concomitant dose of WZ tablet can significantly increase the FK506 whole blood concentration with slight change in FK506 tissue distribution.

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