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Study on the Pharmacokinetics of Tacrolimus in Depression Model Rats

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Abstract: Drug metabolism is affected by many social-psychological factors including mood disorder. Organ transplantation recipients usually suffer from depression. The study aims to explore whether depression disorder alters immunosuppresant Tacrolimus (TAC) pharmacokinetic process in depression model rats. Eighteen female Sprague-Dawley (SD) rats were randomized into model group and control group. Depression model rats were built with Chronic Unpredicted Mild Stresses (CUMS) for 8 weeks. After 8 weeks model establishment, all rats in both groups were given TAC (1.5 mg kg^{-1}) i.g. Blood samples were collected at various time points. TAC concentration was assayed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). TAC pharmacokinetic parameters were processed with DAS software. Compared to those in control group, there was significant decrease in C_{max} and AUC in depression model group ($p<0.05$), from 0.87±0.06 to 0.79±0.05 μg mL^{-1} (C_{max}) and from 2.35±0.23 to 2.14±0.08 μg mL^{-1}•h (AUC), respectively. The results indicated that depression disorder alter TAC pharmacokinetic process which might be induced by enhancing the drug metabolism.

Key words: Pharmacokinetics, tacrolimus, depression, rat

INTRODUCTION

Although organ transplantation offers a significant survival advantage and improve the quality of life of many organ failure patients, most recipients experience a variety of psychological complications such as anxiety, depression, fantasies about the donor, feelings of guilt for the donor and gratitude to the donor's family, etc (Baranyi *et al*., 2013; Dolgoff-Kaspar *et al*., 2012; Reimer *et al*., 2002) and required special psychological adjustment (Dobbels *et al*., 2008). The document showed that the prevalence of depression in kidney transplant recipients is one-fifth, which means a high risk of clinically significant depression (Szeifert *et al*., 2010).

Tacrolimus (TAC) is a potent immunosuppressant which has been widely used alone or in combination with multiple drugs to prevent acute rejection and improve long-term graft survival after solid organ transplantation (Bowman and Brennan, 2008). Due to its narrow therapeutic index TAC is quite easy to induce toxicity or to fail immunosuppression. Therapeutic drug monitoring therefore is standard clinical practice to control effectively the drug blood level in transplant recipients (Staatz and Tett, 2004). Currently the impact of social psychological factors in clinical medication arise the wide interest. Drug efficacy is determined by multiple factors including drug, patient's age and gender, disease, physiological and pathological status, psychological status and others (Xu *et al*., 2008). As a matter of fact it is a common phenomenon in clinical practice that depressive disorder affects the efficacy of drugs (Xu, 2007). Psychological stress may significantly alter pharmacokinetics (Duan *et al*., 2012; Peng and Cheung, 2011). In this study, the impact of depression disorder on the pharmacokinetics of TAC in depression model rats was speculated.

MATERIALS AND METHODS

Establishment of chronic unpredictable mild stress (CUMS)-induced depression model (Katz *et al*., 1981; Willner *et al*., 1987): Female Sprague-Dawley (SD) rats, weighing 180±20 g, 90 days old, were purchased from Laboratory Animal Center of Southern Medical University (Animal Experiment License: SCXK 2006-0015). All animal experiments were in compliance with the Use of Laboratory Animals of National Institutes and the protocol was approved by the Bioethics Committee of

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Southern Medical University. Rats were raised in 25°C experimental lab with normal diet and adapted feeding environment for a week before starting experiment. Eighteen rats were randomized into two groups: model group and control group.

Rats in CUMS-induced depression model group were single cage bred, exposed with random stressor for 8 weeks. These random stressors included heat stress (45°C, 5 min), ice water swimming (4°C, 5 min), tail cliping (1 min), empty cylinder stay (1 h), cages tilting (45°, 24 h), damp padding (10 h) and day/night reversing (24 h). One kind of stressor was random assigned daily; however, the same stressor can't be applied continuously in order to avoid rat's prediction. Each stressor was applied more than 6 times. In the experimental process, depression model rats were moved into another breeding room to accept the stressor (the lighting levels and temperature of the two room were basically the same) and returned after accepting the stressor. The control group rats were single cage bred without any stressor.

Open-field test (Kennett *et al.*, 1985): The apparatus for the open-field test was a square (76×76 cm) made of opaque materials which the open-field arena was partitioned into 25 equal-size squares and surrounded by high walls (42 cm). The test was preceded in a quiet room in the morning (8:00~12:00 am). Each rat was placed in the center of the field and its behavior recorded for 5 min. Four claws climbing square numbers and rearing times were monitored as an index of locomotor activity and exploratory behavior. The open-field was cleaned after each test.

Determination of 5-HT plasma level (Schafer *et al.*, 2010): About 1 mL of blood was collected from each group rat before and after depression model establishment. The anticoagulated blood sample was centrifuged at 3000×g for 5 min to obtain plasma. The level of 5-HT in plasma was tested by Enzyme-linked Immunosorbent Assay (ELISA).

Dosage regimen and blood sample collection: After depression model establishment, all rats were given TAC (1.5 mg kg^{-1}) per os. TAC was supplied kindly by Astellas Pharma Co. Ltd, China. The blood samples were collected at different time and drug concentrations were assayed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The time-point for blood sample collection was at: 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 h.

Determination of TAC concentration in blood (Seger *et al.*, 2009): A modified HPLC-MS/MS assay was used for TAC determination. Briefly, a volume of 50 μL of the internal standard (400 μg mL^{-1}) and 0.45 mL acetonitrile were added to a centrifuge tube, in which 100 μL whole-blood sample was placed. The sample was vortex-mixed vigorously for 1 min, followed by centrifugation at 16000×g for 10 min. Then a volume of 1 μL of the supernatant was directly injected into the HPLC-MS/MS system. HPLC-MS/MS instrumentation and conditions for determination of TAC were: The LC system was consisted of an Agilent 1200 series G1310A pump, a G1310A degasser, a G1329A auto-sampler and a G1316A adjustable column temperature box. The chromatography of the analyte was performed using an Agilent ZORBAX SB-C18 (2.1×150 mm, 5 μm) column. The flow rate of mobile phase (methanol: 0.1 mol L^{-1} ammonium formate, 95:5(v/v)) was 0.4 mL min^{-1}. The optimized conditions of MS/MS with electrospray were as follows: Ion spray source temperature at 350°C, nebulizer (NEB) gas at 10 L min^{-1}, Ion Spray voltage (IS) at 4000 V. For TAC Dissociating Potential (DP) was at 175 V and Collision Energy (CE) was 18 units, for olanzapine Dissociating Potential (DP) was at 140 V and Collision Energy (CE) was 28 units. The mass spectrometer was interfaced to a computer workstation running Aria® OS software and Analyst software (Version B.01.03 Applied Biosystems) for data acquisition and processing. Data acquisition was performed via Multiple-reaction Monitoring (MRM). The ions representing the $[M+H]^+$ species for both the sample and internal standard were selected in MS1 and dissociated (collision-induced) with nitrogen gas to form specific product ions which were subsequently monitored by MS2. The precursor-to-product ion transitions monitored for TAC and olanzapine were m/z 821.6→768.5 and m/z 313→256, respectively.

Statistical analysis: Statistical analysis was performed with the SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and DAS 2.1 software (Drug and Statistics, Shanghai University of Traditional Chinese Medicine, Shanghai, China). Data were expressed as Mean±SD. $p<0.05$ was considered statistically significant.

RESULTS

Validation of CUMS-induced depression model: The locomotion and exploratory behavior scores of rats in depression model group and control group before and after 8 weeks' model establishment were monitored through open-field test. Within each group, the locomotion and exploratory scores of rats in depression model group before and after model establishment were from 78.67±6.91 to 15.22±4.71, from 15.89±2.80 to 4.89±1.69, respectively which were approximately 80 and 70% decrease. However, there were no significant change for the locomotion and exploratory scores in rats of

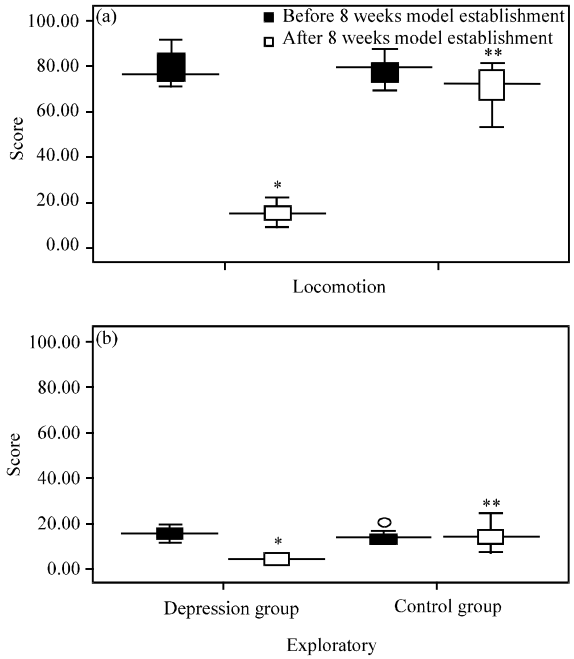

Fig. 1(a-b): Results of open-field test (n = 9 for each group) (a) Locomotion score of rats in depression model group and in control group before and after 8 weeks' model establishment (b) Exploratory score of rats in depression model group and in control group before and after 8 weeks' model establishment, *p<0.01 before and after model establishment score within-group comparison, **p<0.01 after model establishment score between-group comparison

control group before and at the end of 8 weeks, which were from 77.22±6.10 to 70.89±8.91, from 14.67±2.87 to 15.22±5.52, respectively. Meanwhile between the groups, there was no significant difference for locomotion and exploratory scores before the model establishment but significant at the end of 8 weeks (Fig. 1).

Serotonin (5-HT) plasma level was measured by ELISA. The levels of rats in depression model group before and after 8 weeks' model establishment were from 6.74±2.64 to 2.22±0.75 ng mL^{-1} and in control group were from 5.60±1.76 to 5.59±2.09 ng mL^{-1} ($p>0.05$). There was a significant decrease of 5-HT in depression model group before and at the 8 weeks end but no significant difference in control group. Meanwhile, significant difference occurred for 5-HT levels between the model group and the control group at the 8 weeks' end (2.22±0.75 vs. 5.59±2.09 ng mL^{-1}) (Fig. 2).

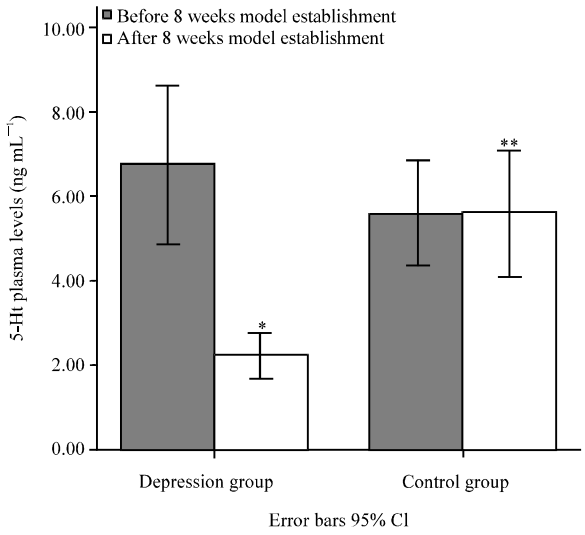

Fig. 2: 5-HT plasma levels in rats of depression group and in control group (n = 9 for each group), *p<0.01 before and after model establishment score within-group comparison. **p<0.01 after model establishment score between-group comparison

Determination of TAC in whole blood: TAC whole blood concentration was determined by HPLC-MS/MS. There was no significant interference or ion suppression from endogenous substances observed at the retention time of the analytes. The retention times for TAC and olanzapine were 1.2 and 1.0 min, respectively (Fig. 3, 4). The calibration curve obtained by weighted linear regression ($1/\chi^2$) showed good linearity over the concentration range from 0.01 to 2.5 μg mL^{-1}. The lowest limit of quantitation was 0.01 μg mL^{-1}. The recoveries of 3 norminal concentrations were about 80%. The accuracies were from 90~100%. The matrix effects were from 88~94%. The intraday variation was less than 7% while the the interday variation was less than 11% (Table 1).

Pharmacokinetics of TAC: TAC blood concentration-time data were analyzed with DAS 2.1 software (Drug and Statistics, China) (Fig. 5, Table 2). There were significant differences in two main pharmacokinetic parameters of TAC between two groups ($p<0.05$). Cmax and AUC of TAC in depression model group were significantly decreased from 0.87±0.06 μg mL^{-1} and 2.35±0.23 μg mL^{-1}•h to 0.79±0.05 μg mL^{-1} and 2.14±0.08 μg mL^{-1}•h, respectively. Although MRT was shortened by nearly 10% in depression model group. It had no significant difference compared to that in control

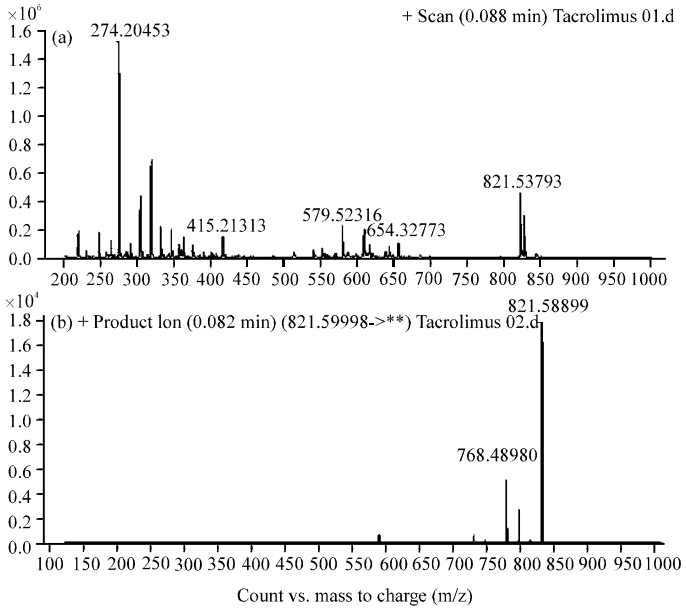

Fig. 3(a-b): Full-scan positive ESI-MS/MS production spectra (a) The $[M+H]^+$ species of TAC, (b) A species product ions of TAC

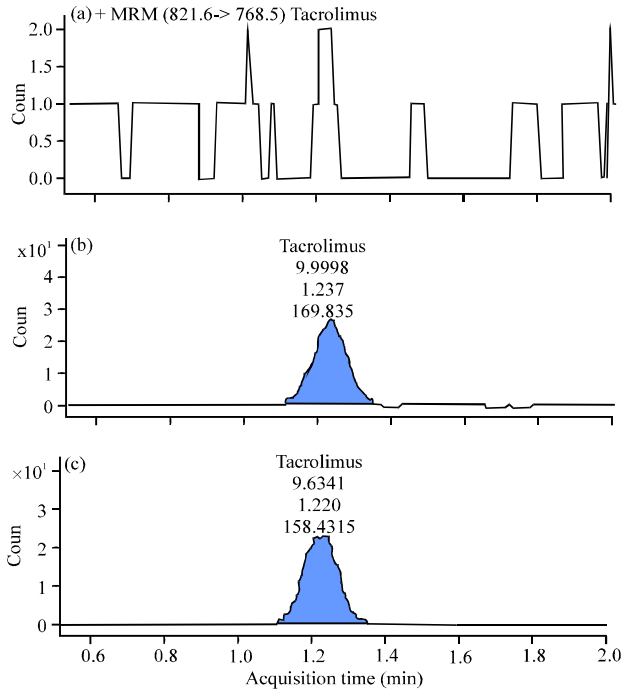

Fig. 4(a-c): HPLC chromatograms of TAC: (a) Blank whole blood sample; (b) A calibration standard containing 1 μg mL^{-1} and (c) A rat whole-blood sample 0.75 h after a single dose of TAC with 1.5 mg kg^{-1}

Table 1: Methodology evaluation for TAC determination assayed by HPLC-MS/MS

Nominal concentration (μg mL^{-1})	Recovery (%)	Measured concentration (μg mL^{-1})	Accuracy (%)	Matrix effect (%)	Intra-day variation (RSD%)	Inter-day variation (RSD%)
2	78.21±2.42	2.006±0.076	100.32	92.48±0.74	4.09	7.38
1	79.06±2.90	0.901±0.022	90.07	93.71±2.20	2.62	2.82
0.02	79.26±3.60	0.019±0.001	98.11	87.98±4.91	6.38	10.73

Data are based on analysis of six replicates (n = 6) on three separate days

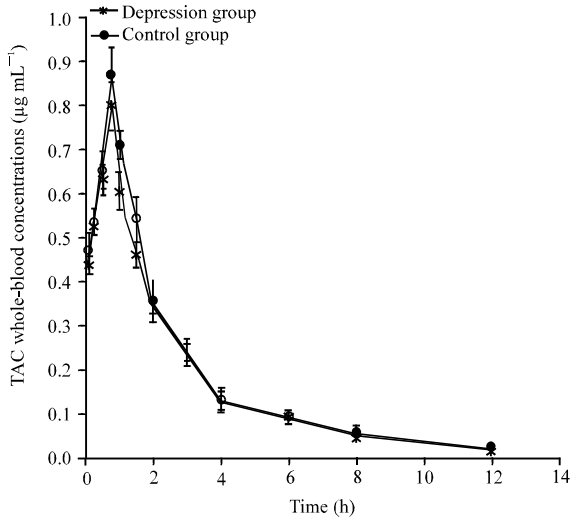

Fig. 5: Blood concentration-time curves of TAC in two groups (each, n = 9) receiving a single dose of TAC (1.5 mg kg^{-1})

Table 2: Pharmacokinetic parameters of TAC of rats in depression model group and in control group

Parameters	Depression group	Control group
$T_{1/2}$ (h)	2.11±0.20[#]	2.23±0.19
T_{max} (h)	0.74±0.19[#]	0.79±0.17
C_{max} (μg mL^{-1})	0.79±0.05[Δ]	0.87±0.06
$AUC_{0-\infty}$ (μg mL^{-1}•h)	2.14±0.08[Δ]	2.35±0.23
MRT (h)	3.20±0.30[#]	3.55±0.50

[#]p>0.05, [Δ]p<0.05 compared with control group

group (p = 0.112). Meanwhile, $T_{1/2}$ and Tmax in depression model group remained almost the same compared to those in control group (p>0.05).

DISCUSSION

An ideal animal model should be able to simulate the development process of diseases and the change of pathophysiology. CUMS is a well-validated animal model which has been used widely for evaluating antidepressant effects for many years (Papp *et al*., 1991; Willner *et al*., 1987). The depressive state in this model rats is similar to change of psychomotor and loss of interest/pleasure in the clinical diagnosis of depression patients. Rats showed a decrease in consumption of sucrose solution, degradation of sexual behavior (Willner, 1997) and changes in plasma 5-HT and NE levels (Liu *et al*., 2011; Spasojevic *et al*., 2009). In this experiment CUMS-induced depression model was verified both by open-field test and by determination of 5-HT plasma levels. The decrease of 5-HT levels was corresponded with the material basis of depression neurobiology. Female rats were adopted in this model, since female ones are more vulnerable to stressors than male counterparts (Kennett *et al*., 1986).

TAC blood concentration was assayed by modified HPLC-MS/MS (Seger *et al*., 2009). Our data shows that the analysis method was stable, accurate and precise which is suitable for this research.

Currently many researches concern about the potential relationship between mood disorder and pharmacokinetics. Kagayama *et al*. (1993) found that CYP2D6 gene polymorphism exists in patients with depression. The genotype and phenotype of CYP2D6 had individual differences which were related to personality traits. The hydroxylation capacity of CYP2D6 was associated with the level of anxiety and the degree of socialization (Dorado *et al*., 2007). It was prone for patients who were poor metabolizer of CYP2D6 to occur adverse drug reactions (Shams *et al*., 2006). Our results displayed the significant differences for C_{max} and AUC between depression model group and control group. It could be speculated, from the point of pharmacokinetics, that chronic mild stress depression might change drug absorption, metabolism and elimination. A preliminary study suggested that the total content and activity of CYP450 enzyme of liver in depression model rats were higher than those in control rats (Duan *et al*., 2012) which suggested that chronic stress might alter the expression and activity of some drug-metabolizing enzymes. Further studies are needed to confirm this hypothesis.

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