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A non-invasive CYP3A4 biomarker and body mass index predict cyclosporine dosage requirements in Chinese renal transplant recipients

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An endogenous CYP3A4 biomarker for *in vivo* metabolism of cyclosporine should be useful for optimizing individual dosage. We aimed to investigate if the combined ratio of endogenous 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone (HOM) in urine could be used as an endogenous probe for the prediction of cyclosporine dosage requirements in renal transplant recipients. 54 medically stable kidney transplant recipients participated in this study. Morning spot blood and urine samples were gathered. The multiple regression analysis including urinary HOM and body mass index accounted for 73.1% of variability in blood concentration/dose ratio (C/D) of cyclosporine, in which urinary HOM and body mass index contributed 64.9% and 8.2%, respectively. Based on the present approach, individual dosage regimen of CsA could be acquired without therapeutic drug monitoring and the results showed that all of the observed stable doses of CsA were within the predicted range during different post-operative periods. In summary, there is a significant relationship between endogenous CYP3A4 biomarker (assessed by urinary HOM) and *in vivo* metabolism of cyclosporine in renal transplant recipients. Urinary HOM and body mass index are important predictors of cyclosporine metabolism. Our findings provide clinical implications that the predictive algorithm based on a simple, safe and non-invasive CYP3A4 phenotyping can be anticipated.

1. Introduction

Cyclosporine (CsA), an effective immunosuppressive agent, has been widely used to prevent allograft rejection after organ transplantation. Therapeutic monitoring of CsA has been recommended because of narrow therapeutic window and large inter-individual variability in its pharmacokinetics (Masuda and Inui 2006). CsA is known to be extensively metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5 (Masuda and Inui 2006), thus variations in CsA pharmacokinetics between individuals can partly be ascribed to differences in expression levels and biological activities of these proteins. Over the years, single nucleotide polymorphisms (SNPs) for CYP3A4 and CYP3A5 have been identified (Masuda and Inui 2006; Hesselink et al. 2003), but most CYP3A4 SNPs are unable to fully explain the inter-individual variability in CYP3A4 enzymatic activity (Oleson et al. 2010). On the other hand, many previous studies have focused on the *CYP3A5*3* allele (A6896G) in exon 3, which encodes the CYP3A5 protein (Kuehl et al. 2001). However, the inference from these data is that *CYP3A5*3* genotypes are not related to inter-individual variability in the disposition of CsA (Hesselink et al. 2003; Burckart and Liu 2006).

Accordingly, the measurement of CYP3A4 phenotype has been suggested as an attractive approach to predict the optimal dosage range of drugs (Zaigler et al. 2000). Paine et al. (2000) reported that *in vivo* CYP3A4 activity reflected by midazolam clearance could explain 25% of variability in CsA clearance. Nowadays, however, endogenous biomarkers for CYP3A4 activity have been paid more attention because of unnecessary administration of probe drugs to humans, thus avoiding any possible physiological changes resulting from the probe drug. In addition, an endogenous probe is the simplest and safest way to sequentially observe *in vivo* enzyme activity over time in individuals and the pharmacological effects of probe drugs cannot be considered as well. It has been suggested that the ratio of 6β-hydroxycortisol to free cortisol (6β-OHF/F) in urine is a truly non-invasive marker of both the induction and the inhibition of CYP3A4 activity (Galteau and Shamsa 2003). Considering the inter-conversion of F to cortisone (E) and 6β-OHF to 6β-hydroxycortisol (6β-OHE) by 11β-hydroxysteroid dehydrogenase (11β-HSD), the clinical results using cortisol as a CYP3A4 probe may also be confounded due to the regulation of cortisol feedback (Luo et al. 2009; Peng et al. 2011). Thus, the 6β-hydroxylation ratio of cortisol and cortisone should be combined to detect CYP3A4 phenotype in clinical studies. Unfortunately, the association between urinary ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone (HOM) and CsA metabolism has not been well investigated.

We conducted a study in Chinese renal transplant recipients to determine the relationship between urinary HOM and dose-corrected C_0 levels (C/D) of CsA. Based on this

Table 1: Desired target range of CsA concentration, predicted stable dosage range and observed stable doses of CsA during different post-operative periods

Post-operative period	Desired target range of concentration (ng/ml) [a]	Predicted dosage range (mg)	Observed dosage (mg)
3-6 months (n = 5)	110-300	96± 42-263± 115	174± 25
6-24 months (n= 13)	100-250	96± 44-241± 111	200± 100
> 24 months (n= 45)	90-250	98± 38-273± 106	149± 46

[a] The desired target range of concentration was set according to the clinical practice in the 3rd Affiliated Hospital of Xiangya Medical Institute, Central South University;
Data: mean ± SD.

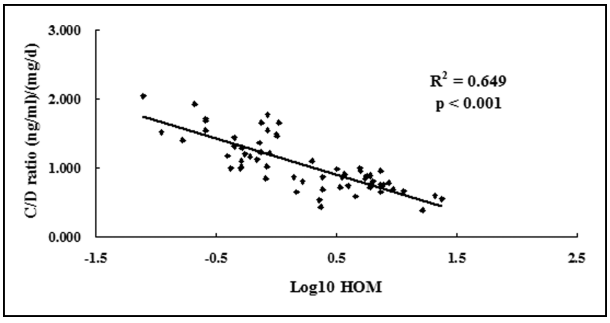

Fig. 1: The relationship between urinary HOM and the C/D ratio of cyclosporine in Chinese renal transplant recipients (n= 63).

potential relationship, individual dosage regimens of CsA could be acquired, taking into account other determinants of CsA disposition.

2. Investigations and results

As shown in the Fig. 1, a significant relationship between urinary HOM and the C/D ratio of CsA was found. The factors that correlated significantly with the C/D ratios of CsA consisted of urinary HOM, height, body mass index (BMI) and alanine aminotransferase (ALT). All these factors were selected to establish the equation for predicting stable doses of cyclosporine by multiple regression analysis. The final regression model included urinary HOM and BMI with Eq. (1) as follows, and the coefficient of determination (R^2) was 0.731 ($p < 0.001$):

$$\text{Stable dose} = C_0/(0.354 - 0.672 * \text{Log}_{10}\text{HOM} + 0.040 \times \text{BMI}) \quad (1)$$

C_0: Trough concentration of CsA; Log_{10}HOM = the logarithm of the ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urine; BMI = body mass index.
According to the desired target range of C_0 levels of CsA, individual dosage regimens could be directly calculated by Eq. (1). Table 1 shows that all of the observed stable doses of CsA were within the predicted range during different post-operative periods.

3. Discussion

According to previously reported data (Hesselink et al. 2003; Burckart and Liu 2006), most pieces of genetic information are not critical determinants of CsA disposition. Of importance, genotyping for *CYP3A5*3* alone could not improve the clinical outcome of CsA therapy. Hence, an endogenous CYP3A4 biomarker for *in vivo* metabolism of CsA should be useful for optimizing individual dosing.
In our study, we first investigated if the C/D ratios of CsA would be predicted using an endogenous CYP3A4 probe in Chinese renal transplant recipients. Taking into account other potential factors, multiple regression analysis was used to predict stable dosage of CsA. Besides urinary HOM, the final model also included individual body mass index, and the coefficient of determination (R^2) was 0.731. Recently, Paine et al. (2000) found that *in vivo* CYP3A4 activity assessed by apparent oral midazolam clearance could explain 25% of variability in oral CsA clearance. By comparison, the utility of endogenous CYP3A4 probe in clinical practice is attractive due to no probe drug administration or serial blood sampling for pharmacokinetic analysis. Our results demonstrated that urinary HOM as a biomarker for CsA metabolism explained 64.9% of variability in dose-corrected C_0 levels of CsA (Fig. 1), and BMI contributed by additional 8.2% as well. The present non-invasive assay explained a total of 73.1% of variance in CsA pharmacokinetics, much greater than previously reported methods based on genotyping and midazolam clearance (Burckart and Liu 2006; Paine et al. 2000). The remaining 26.9% might be attributed to intestinal first-pass metabolism or other unknown factors that were not comprised here.
To date, monitoring of CsA concentration in blood is thought be a routine tool that allows the adjustment of individual dosage. On the basis of concentration-controlled dosing, this approach can help to improve therapeutic efficacy and minimize adverse effects (Masuda and Inui 2006). However, frequent monitoring of drug concentration may be time and cost consuming. Besides labor intensive, therapeutic drug monitoring is also unable to predict optimal initial doses and as a result many patients may have trough concentrations below or above the desired target range in the early post-operative period (Masuda and Inui 2006). At the present study, based on the desired target range of CsA concentration, the predicted dosage range of CsA was obtained by the regression equation and all of the observed doses were within the predicted range during different

Table 2: The data of renal transplant recipients.

Parameter	Value
Age (y)	42.7± 8.9
Gender (male/female)	37/17
Body weight (kg)	60.0± 8.3
Height (cm)	162.7± 9.8
Body mass index (kg/m^2)	22.8± 4.2
Postoperative periods (h)	35638.5± 24128.3
Glutamic-pyruvic transaminase (U/L)	21.7± 19.4
Glutamic oxalacetic transaminase (U/L)	22.7± 13.0
Total bilirubin (μmol/L)	17.1± 5.7
Blood urea nitrogen (mmol/L)	6.3± 2.1
Serum creatinine (μmol/L)	93.5± 36.7
Serum uric acid ((μmol/L))	355.2± 100.2
Comedication of calcium antagonists (with/without)	18/36

Data: mean ± SD.

post-operative periods, indicating an effective prediction of CsA dosage requirements (Table 1).

The major limitation of this study was that a population of Chinese adult renal transplant recipients was at least three months after transplantation. As a result, only stable doses of CsA could be investigated and further studies should focus on the prediction of initial dosage requirements and CsA pharmacokinetics in the early post-operative period, especially a first few critical days after transplantation.

In conclusion, our results confirm that there is a significant relationship between endogenous CYP3A4 biomarker (assessed by urinary HOM) and *in vivo* metabolism of CsA. Combination of body mass index, the present approach based on urinary HOM can be used to predict individual dosage regimens of CsA. As a result, routine monitoring of drug concentrations may no longer be the only method to establish rational treatment with CsA. Our study is the first try to establish the regression relationship with CsA dosage requirements using a simple, low-cost and easily acquired probe with no toxicity and the findings here provide clinical implications that the predictive algorithm based on non-invasive CYP3A4 phenotyping and body mass index can be anticipated.

4. Experimental

4.1. Ethical statement

This study was performed in accordance with the declaration of Helsinki and its amendments. The experimental protocol was approved by the Ethical Committee of School of Pharmaceutical Sciences, Central South University. The study was a part of registered clinical trial in ClinicalTrials.gov and the identifier was NCT01699360 (http://clinicaltrials.gov/show/NCT01699360). Written informed consents were obtained from all subjects before commencing the study.

4.2. Materials

6β-OHF, 6β-OHE, F, E, DM were purchased from Sigma-Aldrich (St Louis, MO, USA). They were of at least 98% purity. Acetonitrile, methanol and formic acid were of LC grade, purchased from Tedia (Tedia Company Inc, Fairfield, USA). All other chemicals were of AR grade available from commercial sources (Sinopharm Chemical Reagent Co. Ltd, Shanghai, China).

4.3. Human subject study

The study comprised 54 medically stable kidney transplant recipients who were at least 3 months post-transplant and 63 data of specimens were collected in Zhangzhou municipal hospital of Fujian province and the 3rd Affiliated Hospital of Xiangya Medical Institute, Central South University. Patients meeting the following criteria were included: receiving an immunosuppressive regimen containing CsA (cyclosporine soft capsules, Sandimmun Neoral®, Novartis, Beijing, China), Mycophenolate Mofetil (CellCept®, Roche, Shanghai, China) and prednisolone; oral administration of CsA with the twice daily dose at least five days prior to the study; patients who had undergone their first renal transplantation; patients who have normal liver and renal function and patients being 18 years and older. The exclusion criteria were as follows: an acute rejection episode or infection; multiple organ transplantation; taking any other medications known to interact with immunosuppressive agents, with exception of calcium-channel blockers; abnormal findings on physical examination or laboratory tests. The demographic data of renal transplant recipients were shown in Table 2.

At the time of investigation the dose of CsA ranged between 50 mg/day and 500 mg/day. On the morning of the study day, morning spot urine specimens were collected at 08:00am-10:00am time intervals. Blood samples were obtained before CsA administration. Immediately thereafter, blood samples were sent to the clinical laboratory for cyclosporine analysis, while urine samples were stored at -20 °C pending analysis for 6β-OHF, 6β-OHE, F and E. All subjects were examined by routine laboratory tests, including hematology, blood chemistries and urinalysis. The demographics of each subject as well as their comedications were also recorded (Table 2). Each patient was instructed to eat his or her usual breakfast, excluding caffeine- and grapefruit-containing foods, and to take any other morning medication, except CsA.

4.4. Analytical methods

4.4.1. 6β-OHF, 6β-OHE, F and E in urine

β6(-OHF, *β6*(-OHE, F and E in urine were measured using the HPLC-UV method developed in our laboratory (Zheng et al. 2015). The lower limit of quantitation was 5 $\mu g \cdot L^{-1}$ for all compounds. Accuracy determined at three concentrations ranged between 98.2% and 115.5%.

4.4.2. Trough concentrations of cyclosporine in whole blood

Trough concentrations of CsA in whole blood were assayed by fluorescent polarization immunoassay (FPIA; Axsym Plus, Abbott, USA), using blood samples taken early in morning. The assay was performed according to the instructions supplied with the kit (Abbott, USA).

4.5. Data and statistical analysis

All statistic analysis was performed by using SPSS 17.0 statistics software and *P* value less than 0.05 was considered significant. The relationship between urinary HOM and the C/D ratios of CsA was analyzed by linear regression. Correlations of the C/D of CsA with each factor (as shown in Table 1) were analyzed with α=0.05 as the size of a test. Factors that were not statistically significant were rejected. A stepwise multiple regression analysis with statistically significant factors was used to obtain coefficient of determination (R^2). The model for the prediction of stable dose of CsA was constructed based on the multiple regression equation.

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Conflict of Interest: The authors declare that they have no conflicts of interest to disclose.

Author contribution: Ningfang Cai, Zeneng Cheng and Xi Luo conceived and designed research; Bifeng Li, Xiaohong Huang, Kezhen Xu, Huiping Feng and Lijun Zhu analyzed clinical samples; Liyun Zheng performed data analysis; Ningfang Cai and Xi Luo wrote the paper.

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