Original Article

Measurement of Whole Blood Tacrolimus Level by High Performance Liquid Chromatography Tandem Mass Spectrometry in Renal Transplant Recipients — A Single Center Perspective

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Background and Methods: Measurement of whole blood tacrolimus level by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was compared with that measured by microparticle enzyme immunoassay (MEIA) in 30 renal transplant recipients.
Results: Whole blood tacrolimus concentrations measured by HPLC-MS/MS were significantly lower than those measured by MEIA, with a median difference (interquartile range, IQR) of –0.40 μg/L (2.03 μg/L) ($p < 0.0005$). MEIA overestimated tacrolimus concentrations by a median (IQR) of 5.04% (19.5%). There was good correlation between the two methods ($r = 0.94$; $p < 0.0005$). The Passing-Bablok regression equation was: HPLC-MS/MS (μg/L) = 0.96 (95% confidence interval, CI, 0.91–1.00) × MEIA (μg/L) – 0.02 (95% CI, –0.40–0.46). Bland-Altman analysis showed that the 95% limits of agreement were 2.98 to –4.10 μg/L. The 12-hour area under the concentration curve (AUC_{12}) of tacrolimus derived using the two-point sampling equation with tacrolimus concentrations measured by HPLC-MS/MS was compared with that measured by MEIA. The AUC_{12} values calculated by the two methods were highly correlated ($r = 0.90$; $p < 0.0005$). The mean difference between the AUC_{12} values was 3.4 ± 11.6 hr.μg/L, and the mean percentage difference was 2.6 ± 11.4%, both of which were not statistically significant.
Conclusion: For tacrolimus concentrations within the recommended therapeutic range, the concentration measured by HPLC-MS/MS was statistically significantly lower than that measured by MEIA, but the difference was not clinically significant. Introduction of the more specific HPLC-MS/MS method does not require adjustment in the recommended tacrolimus trough concentration or the AUC_{12} level estimated by our abbreviated regression equation. [*Hong Kong J Nephrol* 2005;7(2):65–9]

Key words: drug concentration, HPLC-MS/MS, MEIA, kidney transplant, tacrolimus

背景與方法：本研究對 30 位腎臟移植接受者，分別以兩種方法進行 tacrolimus 濃度的測量：高效能液相層析串聯質譜儀分析 (HPLC-MS/MS) 、及微粒酵素免疫分析法 (MEIA)，並對兩者作出比較。
結果：HPLC-MS/MS 測量所得之全血 tacrolimus 濃度顯著低於 MEIA 的測量結果，差異中位數為 (四分位數間距，IQR) –0.40 μg/L (2.03 μg/L) ($p < 0.0005$)；MEIA 高估 tacrolimus 濃度達中位數 5.04% (IQR 則為 19.5%)。兩種測量法的結果間存在著良好相關性 ($r = 0.94$；$p < 0.0005$)，Passing-Bablok 迴歸方程式為：HPLC-MS/MS (μg/L) = 0.96 (95% CI，0.91 to 1.00) × MEIA (μg/L) –0.02 (95% CI，–0.40 to 0.46)；Bland-Altman 分析顯示 95% 一致區間為 2.98 至 –4.10 μg/L。此外，研究人員以兩點取樣方式，計算出 12 小時濃度曲線下面積 (AUC_{12}) 作為比較的指標，亦發現 HPLC-MS/MS 與 MEIA 間的高度相關性 ($r = 0.90$；$p < 0.0005$)。兩者間的 AUC_{12} 平均差異為 3.4±11.6 hr.μg/L 或 2.6±11.4%，並未達到統計學意義。
結論：在建議劑量範圍內使用 tacrolimus，HPLC-MS/MS 測量所得之藥物濃度顯著低於 MEIA 所

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得的結果，但此差異未達臨床意義。採用高特定性之 HPLC-MS/MS 為測量方法後，tacrolimus 的建議血濃度波谷值 (trough level) 及以兩點取樣方式計算的 12 小時濃度曲線下面積 (AUC_{12}) 並不需要作出調整。

Introduction

Tacrolimus is a potent immunosuppressive agent used in solid organ transplantation. It has a relatively narrow therapeutic index [1]. The correlation of dosage to its blood concentration is poor as a result of variability in pharmacokinetic parameters among patients [2]. A consensus has been made that therapeutic monitoring of whole blood tacrolimus concentration at steady state is required in view of its dose-related efficacy and toxicity, narrow therapeutic index, possible cytochrome P450 mediated drug interactions, and considerable interpatient variability in its pharmacokinetics [3]. Microparticle enzyme immunoassay (MEIA) has been commonly used for the measurement of tacrolimus concentrations in blood as the instrument required is not very expensive and can be found routinely in clinical laboratories [4]. However, MEIA uses an anti-tacrolimus monoclonal antibody that recognizes not only the parent drug but also several of its metabolites. Previous studies have demonstrated that this may lead to overestimation of drug concentration when compared with a method that is specific for the parent drug [4,5].

High performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) is a sensitive and specific method for measuring whole blood tacrolimus concentrations [6]. HPLC-MS/MS has recently been introduced into hospitals in Hong Kong to replace the less specific MEIA. However, our past clinical experience with tacrolimus in renal transplant recipients has been based on tacrolimus levels measured by MEIA. To determine if the change in assaying method would affect our recommended drug dosages for renal transplant recipients, we compared the results obtained by the new HPLC-MS/MS method against the previously established MEIA assay results in the clinical setting of a group of stable renal transplant recipients in our center. We examined whether the change in measurement methods would affect the clinical management of these patients, especially regarding whether or not there is a need to develop an assay-specific target blood tacrolimus concentration range in the maintenance immunosuppressive therapy of renal transplant recipients.

Methods

Thirty renal transplant recipients receiving tacrolimus were recruited into the study. Their standard immunosuppressive regimen included tacrolimus, prednisolone (7.5 mg/day) and azathioprine (1.5 mg/kg). To compare the analytical performance of the two assay methods, 134 blood samples from these patients were collected by venipuncture and sent to the laboratory in ethylenediaminetetraacetic acid (EDTA) tubes. The whole blood tacrolimus concentrations of each blood sample were measured by both MEIA and HPLC-MS/MS.

To compare the effects of the two analytical methods on clinical management, 50 pairs of 2-hour post-dose (C2) and 4-hour post-dose (C4) whole blood tacrolimus concentrations from the recruited patients were used. Estimation of the 12-hour area under the concentration curve (AUC_{12}) for tacrolimus was done by using a two-point sampling method with the equation, $AUC_{12} = 16.2 + 2.4 \times C2 + 5.9 \times C4$, that was previously validated by our group using tacrolimus concentrations as measured by MEIA [7]. The AUC_{12} derived from tacrolimus concentrations measured by HPLC-MS/MS using the same equation was then compared with the AUC_{12} derived from tacrolimus concentrations measured by MEIA.

MEIA

MEIA was performed on an IMx System analyzer according to the manufacturer's instructions (IMx Tacrolimus II assay; Abbott Diagnostics, Abbott Park, IL, USA). In short, a whole blood sample was extracted with a precipitation reagent and centrifuged. Tacrolimus in the supernatant competed with tacrolimus-alkaline phosphatase conjugate for antibodies coated on microparticles. An aliquot of the reaction mixture containing the tacrolimus or conjugate bound microparticles was transferred to a glass fiber matrix; the microparticles bind irreversibly to the glass fiber matrix. The matrix was washed to remove unbound materials. A fluorogenic substrate for alkaline phosphatase was added to the matrix and the fluorescent product was measured by the optical assembly. The rate of fluorescence production is inversely related to the concentration of tacrolimus in the test sample. Between-run coefficients of variation were 4.2% at 19.7 μg/L and 9.7% at 6.2 μg/L. The limit of detection was 1.5 μg/L.

HPLC-MS/MS

The HPLC-MS/MS method was performed on the Agilent 1100 HPLC system (Agilent Technologies Inc, Palo Alto, CA, USA) equipped with the Sciex API 2000 MS/MS detector (Applied Biosystems, Foster City, CA,