DEVELOPMENT OF AN LC-MS/MS METHOD TO DETERMINE TACROLIMUS AND EVEROLIMUS IN KIDNEY TISSUES AND ITS APPLICATION TO KIDNEY TRANSPLANT RECIPIENTS

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論文題名　：DEVELOPMENT OF AN LC-MS/MS METHOD TO DETERMINE TACROLIMUS AND EVEROLIMUS IN KIDNEY TISSUES AND ITS APPLICATION TO KIDNEY TRANSPLANT RECIPIENTS （LC-MS/MS 法を用いたタクロリムスおよびエベロリムスの腎組織中濃度測定方法の確立と腎移植患者への応用）

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論　文　内　容　の　要　旨

Background

The calcineurin inhibitor (CNI) tacrolimus has been widely used in immunosuppressive therapy after kidney transplantation. However, avoiding CNI-induced nephrotoxicity remains an unsolved challenge in clinical practice. Everolimus, a potent mammalian target of rapamycin (mTOR) inhibitor, its major feature compared to tacrolimus is the lower risk of nephrotoxicity and thus provides more options for immunosuppressive treatment. Although the therapeutic drug monitoring (TDM) of tacrolimus and everolimus whole blood trough concentrations (C_{blood}) have been widely performed in clinical practice, the incidence of adverse events remains difficult to predict and prevent. Some studies have suggested that directly measuring the drug concentrations in the target location could be a more effective approach in drug monitoring. To the date, allograft however, no study has simultaneously determined tacrolimus and everolimus concentrations in allograft kidneys (C_{tissue}) and the clinical relevance of tacrolimus and everolimus C_{tissue} is still unclear.

Tacrolimus is predominantly metabolized by cytochrome P450 (CYP)3A4 and CYP3A5 in intestine and liver. It is well known that the recipient (intestinal and hepatic) loss-of-function allele *CYP3A5*3* is the key genetic factor affecting tacrolimus pharmacokinetics. Patients with recipient *CYP3A5*1* allele (defined as CYP3A5 expressors) require higher tacrolimus doses to achieve target blood concentrations compared to those with recipient *CYP3A5*3/*3*. However, adjusting tacrolimus dose based on the recipient *CYP3A5* genotype did not significantly improve clinical outcomes. As the CYP3A5 protein is also expressed in renal tubular epithelial cells, it is reasonable to assume that donor (allograft kidney) *CYP3A5* genotype might have a closer relationship with clinical outcomes by affecting the local tacrolimus concentration in the kidney.

Therefore, the aims of this study are to develop the first LC-MS/MS method for simultaneously quantifying tacrolimus and everolimus in clinical kidney biopsies; and investigate the clinical value of tacrolimus and everolimus C_{tissue} in kidney transplant recipients.

Method and Results

Chapter 1 Development of an LC-MS/MS method for the determination of tacrolimus and everolimus in kidney biopsy samples

The developed LC-MS/MS method was fully validated according to FDA requirements. The concentrations in kidney homogenate could be measured in range of 0.02-2.0 ng/mL for tacrolimus, and 0.04-4.0 ng/mL for everolimus. This method requires only a simple protein precipitation process and has a run time of 8 min. Tissue samples were stable for at least 6 h at room temperature, 3 months of storage at -80 °C, 3 freeze-thaw

cycles, and 20 h at an autosampler. The developed method was successfully used to measure tacrolimus and everolimus C_{tissue} in kidney transplant recipients, and it revealed that the C_{tissue}/D of tacrolimus and everolimus was significantly associated with their corresponding C_{blood}/D (r = 0.9385, $P < 0.0001$ and r = 0.6659, $P = 0.0113$, respectively)..

Chapter 2 Effect of donor *CYP3A5* gene polymorphism on tacrolimus kidney concentration in kidney transplant recipients

A total of 52 Japanese kidney transplant patients receiving tacrolimus were enrolled in this study. 74 kidney biopsy specimens were obtained at 3 months (n = 52) and 1 year (n = 22) after transplantation to determine the donor *CYP3A5* polymorphism and measure tacrolimus C_{tissue} by LC-MS/MS. The allele frequencies for *CYP3A5*3* in donors and recipients were 71.2% and 74.0%, respectively. The tacrolimus C_{tissue} ranged from 52 to 399 pg/mg tissue (n = 74). Patients with recipient (intestinal and hepatic) *CYP3A5*1* allele showed lower tacrolimus levels both in whole blood and kidney tissue compared to those with recipient *CYP3A5*3/*3* at 3 months after kidney transplantation ($P = 0.0008$ and $P = 0.0096$, respectively). Tacrolimus C_{tissue}/D was significantly correlated with tacrolimus C_{blood}/D at 3 months and 1 year after transplantation (Spearman, r = 0.7604, $P < 0.0001$ and r = 0.7572, $P < 0.0001$, respectively). However, donor *CYP3A5* gene polymorphism had no significant impact on tacrolimus C_{tissue}/D (**Figure 1**).

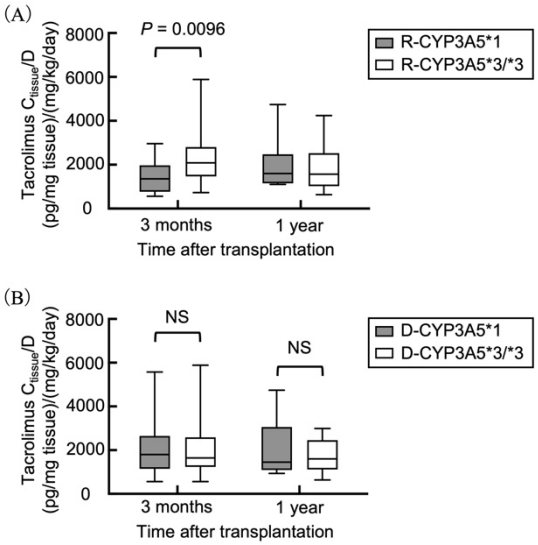

Figure 1. Influence of recipient and donor CYP3A5 genotype on tacrolimus kidney levels.

Discussion

In chapter 1, this is the first study to develop a method for determining tacrolimus and everolimus concentrations in clinical kidney biopsies. The selectivity, linearity, precision, accuracy, stability, recovery, and matrix effect of the developed method were compliant with the requirements of FDA Bioanalytical Method Validation Guideline. The sample preparation procedure was simple and economic; with a high sensitivity, the method has been proven to be suitable for measuring tacrolimus and everolimus in biopsy-sized kidney tissue samples. In chapter 2, the influence of recipient and donor *CYP3A5* polymorphism on tacrolimus concentrations both in whole blood and allograft kidney have been investigated. Tacrolimus allograft kidney exposure were found to be significantly associated with the whole blood levels and recipient *CYP3A5* genotype. There was no impact of donor *CYP3A5* genotypes on the tacrolimus C_{blood} or C_{tissue}, and recipient *CYP3A5* polymorphism seem to play a more important role in tacrolimus kidney accumulation. In conclusion, donor *CYP3A5* gene polymorphism alone cannot be used to predict tacrolimus intrarenal exposure in kidney transplant recipients.