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　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 審 査 の 結 果 の 要 旨

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, 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, the following new findings are obtained.

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).

Next, a total of 52 Japanese kidney transplant recipients receiving tacrolimus were enrolled in the study. seventy-four 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 kidney exposure. There was no significant difference in tacrolimus C_{tissue} between the no rejection and subclinical acute rejection (SubAR) groups of recipients.

The results demonstrate a simple, reliable, and reproducible LC-MS/MS method to allow the quantification of tacrolimus and everolimus concentrations in allograft kidney. 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. The method could support further investigation of the clinical relevance of tacrolimus and everolimus allograft concentrations in kidney transplant recipients. Moreover, 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 was 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 seems to play a more important role in tacrolimus kidney accumulation. The results imply that donor *CYP3A5* gene polymorphism alone cannot be used to predict tacrolimus kidney exposure after kidney transplantation. For the first time, this study investigates the impact of donor *CYP3A5* polymorphism on tacrolimus kidney exposure, the results may be useful for exploring tacrolimus kidney metabolism and minimizing CNI-induced nephrotoxicity in kidney transplant recipients.

以上の結果より、本研究の意義は大きく、博士（臨床薬学）の学位に値すると認める。