Individualized dosing of calcineurin inhibitors in renal transplantation

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Summary

The research presented in this thesis aims to individualize calcineurin inhibitor (CNI) therapy in renal transplantation. Therapy with CNIs, to prevent kidney rejection, has (renal-) tissue deteriorating toxicity as a serious downside. Until other drugs are developed that prevent the use of CNIs or that allow early withdrawal, factors should be identified that provide a way to individualize the dose of the CNIs tacrolimus and ciclosporin A (CsA) in order to maintain the CNI dose as low as possible (**Chapter 1**).

Patient variability in clinical response to CsA and tacrolimus partly results from differences in CNI exposure. For tacrolimus drug interactions and genetic variability relate to tacrolimus exposure. Patients carrying the CYP3A5*1 allele have an increased tacrolimus metabolism, hence lower drug exposure. Adjusting the tacrolimus dose to this genotype is a tool to optimize therapy from a pharmacokinetic perspective. In contrast, no genetic variants have been found to clearly relate to CsA exposure. Despite therapeutic drug monitoring aimed at individualizing CNI therapy, patients still suffer from acute or chronic rejection and CNI toxicity. To further optimize CNI therapy future research may incorporate genetic polymorphisms in proteins involved in CNI pharmacodynamics (i.e. drug target). Proteins potentially relevant for drug response are calcineurin and the CNI binding proteins immunophilins. Moreover, since the expression of the nuclear factor of activated T-cells (NFAT) is reduced after calcineurin inhibition, genetic polymorphisms in the genes encoding NFAT may also be interesting candidates for studying inter-patient differences in CNI efficacy and toxicity. In addition, the existence of isoforms and differences in tissue distribution of the calcineurin protein could potentially explain variable drug response.

At present, the focus has been on the metabolism of CNIs and not on variability in the drug target. Therefore, future improvements in CNI therapy are likely to occur from a systems pharmacology approach taking into account genetic markers for both CNI pharmacokinetics and pharmacodynamics (**Chapter 2**).

To prevent acute rejection episodes it is important to reach adequate tacrolimus exposure early after kidney transplantation. With a better understanding of the high variability in the pharmacokinetics of tacrolimus the starting dose can be individualized, resulting in a reduction in dose adjustments to obtain the target exposure. A population pharmacokinetic analysis was performed to estimate the effects of demographic factors, hematocrit, serum albumin concentration, prednisolone dose, tacrolimus dose interval, polymorphisms in genes coding for ABCB1, CYP3A5, CYP3A4 and the pregnane X receptor on tacrolimus pharmacokinetics. Pharmacokinetic data were prospectively obtained in 31 *de novo* kidney transplant patients randomized to receive tacrolimus once or twice daily and subsequently, the data were analyzed by means of Non-Linear-Mixed-Effects-Modelling. Tacrolimus clearance was 1.5 fold higher for patients with the CYP3A5*1/*3 genotype compared to the CYP3A5*3/*3 genotype (5.5±0.5L/h *versus* 3.7±0.3 L/h respectively). This factor explained 30% of the inter-individual variability in apparent clearance and thus drug exposure. Also, a relationship between the pregnane X receptor A+7635G genotype and tacrolimus clearance was identified with a clearance of 3.9±0.3 L/h in the A-allele carriers *versus* 5.4±0.6L/h in the GG genotype. Finally, a concomitant prednisolone dose of more than 10 mg/day increased the tacrolimus apparent clearance by 15%. In contrast body weight was not related to tacrolimus clearance in this population. As patients are typically dosed per kg body weight this might result in under- and overexposure in patients, with a low and high body weight respectively. This integrated analysis shows that adult renal transplant recipients with the CYP3A5*1/*3 genotype require a 1.5 times higher fixed starting dose compared to CYP3A5*3/*3 in order to reach the predefined target exposure early after transplantation (**Chapter 3**).

In agreement with tacrolimus, optimal CsA exposure in kidney transplant recipients is difficult to attain because of variability in CsA pharmacokinetics. A better understanding of the variability in CsA exposure could be a good means of individualizing therapy. Specifically, genetic variability in genes involved in CsA metabolism could explain exposure differences. Therefore, chapter 4 aimed at identifying a relationship between genetic polymorphisms and the variability in CsA exposure, while accounting for non-genetic sources of variability as well. *De novo* kidney transplant patients ($n=33$) were treated with CsA for 1 year and extensive blood sampling was performed on multiple occasions throughout the year. The effects of the non-genetic covariates hematocrit, serum albumin concentration, cholesterol, demographics (i.e. bodyweight), CsA dose interval, prednisolone dose and genetic polymorphisms in genes encoding ABCB1, CYP3A4, CYP3A5, and PXR on CsA pharmacokinetics were studied using non-linear mixed effect modeling. The pharmacokinetics of CsA was described by a two-compartment disposition model with delayed absorption. Body weight was identified as the most important covariate and explained 35% of the random inter-individual variability in CsA clearance. Moreover, concurrent prednisolone use at a dosage of 20 mg/day or higher was associated with a 22% higher clearance of CsA, hence lower CsA exposure. In contrast, no considerable genotype effects (i.e. greater than 30-50%) on CsA clearance were found for the selected genes. It appears that the selected genetic markers explain variability in CsA exposure insufficiently to be of clinical relevance. Therefore, therapeutic drug monitoring is still required to op-

timize CsA exposure after administration of individualized doses based on body weight and as this study suggest, co-administration of prednisolone (**Chapter 4**).
The CNI trough concentration and serum creatinine monitoring are the current standard biomarkers to assess systemic drug exposure and renal function, respectively. Serum creatinine is a notoriously unreliable marker for GFR; changes in creatinine concentration occur late in disease progression and do not accurately represent the ongoing underlying renal damage (5). Our point is that monitoring the trough concentration without information on the patient's absorption profile or the related systemic drug exposure is equally unreliable for guiding initial CNI dosing or for controlling systemic drug exposure while tapering. Until more sophisticated pharmacodynamic tools become available, advanced TDM with population pharmacokinetics constitutes the preferred CNI intervention strategy to optimize the long-term graft survival of the scarce organs available for transplantation (**Chapter 5**).
Despite therapeutic drug monitoring of ciclosporin A (CsA) blood concentrations, renal transplant recipients still suffer from acute rejection episodes and nephrotoxicity. Insight into the individual susceptibility for CsA therapy is warranted to further individualize therapy. A biomarker such as the activity of calcineurin, the target enzyme of CsA, could potentially reflect the between patient variability in treatment response. Therefore, the pharmacokinetic-pharmacodynamic (PK-PD) relationship between CsA blood concentration and calcineurin activity was evaluated. Renal transplant recipients ($n=98$) were treated with CsA for 6 months after transplantation. CsA blood concentrations and calcineurin phosphatase activity in leukocytes were measured frequently and analyzed using a population PK-PD analysis. The PK of CsA was found to be linear with delayed absorption. The change in calcineurin activity was directly related to the CsA blood concentration and the PK-PD relationship was best described with a sigmoid maximum-effect (E_{max}) model. The baseline activity (E_0) with a median value of 10 pmol/min/mg protein showed considerable within subject variability of 28%, which could be partly explained by differences in intra-cellular protein amount and assay-variability. The E_{max} was 48% of the baseline activity and the CsA potency (IC_{50}) was found to be 223 µg/L, with only a small between subject variability in E_{max} of 13%. Although a clear relationship between CsA blood concentration and calcineurin activity in leukocytes was observed in the population, differences in individual susceptibility for CsA, in terms of efficacy and potency, could not be identified, limiting the usefulness of this biomarker for the individualization of CsA dosing (**Chapter 6**).
Subclinical acute rejection (SCR) in the first year after renal transplantation is associated with early graft loss. Besides, presence of SCR may prevent reduction of immunosuppressive therapy. Therefore, we aimed to identify which factors are predictive for SCR. Specifically, genetic variability in the genes encoding calcineurin (*PPP3CA*/*PPP3CB*) was of interest. Adult renal transplant recipients ($n=361$), receiving quadruple immunosuppression consisting of basiliximab, prednisolone, mycophenolate sodium and ciclosporin A (CsA), were followed for 6 months as part of a multicenter study. At 6 months after transplantation a scheduled biopsy was obtained and reviewed for signs of SCR. Together with demographic and transplant related factors, CsA exposure data ($AUC_{0\text{-}12h}$) and pharmacogenetic data (variability in the genes *ABCB1*, *CYP3A5*, *CYP2C8*, *NR1I2*, *PPP3CA* and *PPP3CB*)

were analyzed with S-Plus/NONMEM. Biopsies were obtained for 275 transplant recipients, of which 18% ($n=50$) displayed SCR. A previous acute rejection episode and a cadaveric donation were the most important predictors for SCR, leading to a risk of 52% of SCR at 6 months (*versus* 11% average), while these factors, along with female sex and carrying ABCB1 TTT-haplotype, were related to a premature end of study (overall drop-out 24%). Genetic variability in the genes (*PPP3CA*/*PPP3CB*) coding for calcineurin were not significantly related tot SCR. Transplant related factors were found to be the most important predictors for SCR in this AUC-controlled population on CsA (**Chapter 7**).

More insight into CNI therapy has been developed. Individualized dosing on basis of CYP3A5*1 genotype is a possible approach for tacrolimus, while body weight is an adequate predictor for ciclosporin exposure. However in both cases concentration monitoring is still necessary to further individualize therapy and to prevent overexposure early after renal transplantation. Major clinical developments in optimizing calcineurin therapy will be related to finding the lowest dose that prevents kidney rejection, but substantially reduces toxicity. With respect to renal toxicity, the main focus should be on genetic factors to be identified in the donor kidney.

Progress in optimizing the pharmacological intervention after renal transplantation is expected to come from three approaches. First of all it embraces the development of biomarkers indicative for the immunological risk as well as drug response biomarkers indicative for the action of the various immunosuppressive drugs together. The latter will include pharmacogenetic markers in the pharmacodynamic pathways of the various drugs (i.e. the *PPP3CA* and *PPP3CB* gene encoding calcineurin). Furthermore, the analysis type should be brought to higher level using pharmacometrics to its full extent. This thesis served to introduce population approaches with non-linear mixed effects modeling in renal transplantation. This should further be exploited by a more comprehensive systems pharmacology approach including biomarkers that reflect adaptation of the immune system to the new situation (transplanted organ, drugs etc.), a derivative of disease-progression models and 3-dimensional response surface analysis to account for the effect of interacting immunosuppressive drugs. Finally, large collaborations should join data to maximize success of the pharmacometric approaches (**Chapter 8**).