Chapter 1

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A number of enzymes in humans are capable of biotransforming lipid-soluble xenobiotics in such a way as to render them more water soluble. There are two types of enzyme reactions: phase I and phase II type reactions. Phase I type reactions are involved in hydrolysis, oxidation and reduction reactions, whereas phase II type reactions have been referred to as conjugative reactions which include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis), or with amino acids (e.g., glycine, taurine and glutamic acid). Although it is well known that not all conjugation reactions are secondary phase II reactions since many endobiotics (e.g. bilirubin, steroids, retinoids, and bile acids) and xenobiotics (e.g. acetaminophen, morphine, and oxazepam) are conjugated without prior conversion to products generated from oxidative, reductive or hydrolytic enzymes. Conjugative biotransformation results in an increase in the hydrophilicity of a compound which promotes its excretion. The balance between the cytochrome P450 and conjugative drug biotransforming enzymes is responsible for either the detoxification or the accumulation of toxic metabolites in the body. The calcineurin inhibitor tacrolimus has highly variable pharmacokinetic characteristics and therefore close therapeutic drug monitoring is required especially to prevent subtherapeutic and toxic blood levels. Sub-therapeutic drug levels increase the risk of rejection significantly while toxic drug levels cause serious side effects, as described in CHAPTER 2. Additionally, this CHAPTER includes a complete review of the pharmacokinetics and pharmacogenetics of tacrolimus. Although several commercial DNA isolation kits are available to extract deoxyribonucleic acid (DNA) from whole blood samples, less information is available regarding the extraction efficiency and the quality of the isolated DNA. CHAPTER 3 presents a study that compares three commercially available DNA isolation kits regarding their ability to extract DNA from whole blood samples as well as the DNA quality. In CHAPTER 4 a locked nucleotide acid (LNA) on the polymorphic position in the sensor probe is introduced. By using a LNA on the polymorphic position, a better discrimination between the wild type allele and the variant allele is possible. The influence of a LNA in a FRET probe and its consequence on the melting curves is described in CHAPTER 4. Due to the introduction of real-time PCR technology it is possible to detect single nucleotide polymorphisms (SNPs) faster, with less contamination risk and less hands on time than previously used PCR methods, like PCR restriction fragment length polymorphism (RFLP) and PCR amplification refractory mutation system (ARMS). CHAPTER 5 reports new real-time PCR fluorescence resonance energy transfer (FRET) assays for SNPs which are compared with PCR RFLP and PCR ARMS assays. CHAPTER 6 evaluates 24 limited sampling strategies for tacrolimus that have been published by other research groups in an independent renal transplant recipient group. This comprehensive study demonstrates that a limited sampling strategy using two (C_2, C_4) or three (C_0, C_2, C_4) sample time points are a better indication for the tacrolimus exposure than the trough (C_0) levels alone. In most

transplant centres monitoring of the tacrolimus blood concentration is performed by C_0 concentrations after the morning tacrolimus administration. However, conflicting evidence is present regarding the usefulness of these morning tacrolimus C_0 concentrations. Knowing this, several studies recently developed a limited sampling strategy for tacrolimus using stepwise multiple regression analysis or Bayesian fitting to predict the AUC_{0-12} as accurate as possible, since the AUC_{0-12} is considered to be the gold standard of measuring the total tacrolimus exposure. Most of these studies that developed a limited sampling strategy for tacrolimus used no independent transplant patient population to validate their limited sampling strategies. Several studies reported an association between the ABCB1 polymorphisms C1236T, G2677T/A and C3435T or ABCB1 haplotypes and the tacrolimus C_0 levels or the pharmacokinetic parameters of tacrolimus in different transplantation patients, while other studies found no correlation between the ABCB1 polymorphisms and the tacrolimus blood levels. In CHAPTER 7 and 8 the influence of CYP3A and ABCB1 polymorphisms is studied in a Caucasian and a Chinese renal transplant recipient group while CHAPTER 9 examines the presence of the CYP3A7*1C variant allele in Chinese and Caucasian renal transplant recipients and investigates the association between the CYP3A7*1C variant allele and the pharmacokinetic tacrolimus parameters. Previously, it was demonstrated that UGT2B7 is the most important member of the UGT family involved in the tacrolimus glucuronidation although the influence of the recently identified UGT2B7 polymorphisms on the tacrolimus blood levels was never reported. CHAPTER 10 reports the contribution of three UGT2B7 polymorphisms on the tacrolimus blood levels in a Chinese renal transplant recipient group. The nuclear receptor pregnane X receptor (PXR) activates transcription as a response to a diversity of natural and synthetic compounds. Recently, it was demonstrated that eight SNPs present in the untranslated and intron regions of the PXR gene showed after induction with rifampicin a significant difference or trend in the erythromycin breath test, in the CYP450 3A located in the human hepatocytes or intestines and in the ABCB1 expression in the intestines. The role of PXR polymorphisms in correlation with a limited sampling strategy for tacrolimus is examined in a Chinese renal transplant recipient group as described in CHAPTER 11. Since it is also known that corticosteroids can induce the CYP3A enzymes, these PXR polymorphisms may also play a role in the tacrolimus metabolism, especially in the early transplant period when transplant patients receive high daily doses of corticosteroids. In CHAPTER 12 the influence of corticosteroids on the contribution of the PXR polymorphisms demonstrates that the role of CYP3A enzymes may be significantly influenced by the combination of an induced PXR gene and the presence of PXR polymorphisms. The corticosteroids activate the PXR gene which as a result induce the CYP3A enzymes that lead to lower tacrolimus blood levels. CHAPTER 13 presents some general conclusions and comments on the obtained results. Future perspectives and recommendations on the different pharmacokinetic and pharmacogenetic approaches used nowadays and possibly in the (near) future and its consequences for transplantation patients in daily practice are also discussed.

This thesis includes three important aims which have been studied:

I A comparison and validation of limited sampling strategies for tacrolimus previously published, which may provide a better indication of the tacrolimus exposure in different transplant patients (CHAPTER 6).

II The introduction of real-time PCR FRET technology that makes genotyping of several SNPs present in different genes possible in a shorter analytical time, with less hands on time, and with less contamination risk compared to the conventional PCR methods (CHAPTER 3, 4 and 5).

III Knowledge on the presence of SNPs in the phase I and phase II enzymes, in transporters and nuclear receptors in the tacrolimus metabolism may contribute to decrease the high inter-individual pharmacokinetic variation in the tacrolimus blood levels of Caucasian and Chinese renal transplant patients (CHAPTER 7, 8, 9, 10, 11 and 12).