CHAPTER 2

Pharmacokinetics and pharmacogenomics of tacrolimus: a review

Submitted in an adapted form

1 Introduction

Tacrolimus (FK506) is a 23-membered macrolide lactone ($C_{44}H_{69}NO_{12}$; for molecular structure see Figure 2.1) which is isolated from the fermentation broth of *Streptomyces tsukubaesis*[1,2]. Additionally, tacrolimus like cyclosporin is an immunosuppressive agent belonging to the calcineurin inhibitor group that has emerged as a valuable therapeutic alternative to cyclosporine following solid organ transplantation[3]. It is highly effective at preventing rejection in heart[4-7], small bowel[8,9], pancreas[10-14], bone marrow[10-14], lung[15-19], liver[20-24], and kidney[25-28] recipients and for the therapy of autoimmune diseases[29,30].

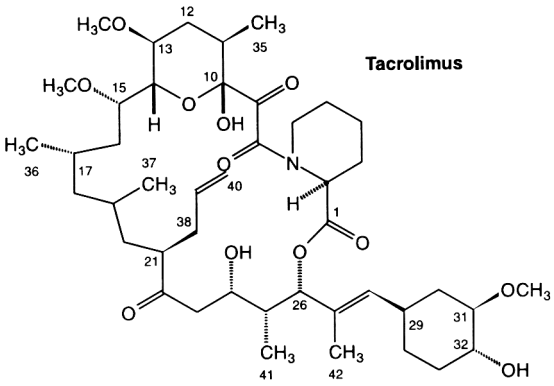

Figure 2.1 Structure of tacrolimus.

Although there are structural differences, cyclosporin and tacrolimus share a similar cellular mechanism of action, though tacrolimus, is 10 to 100 times more potent at the molecular level[31]. After entry into the cell, both agents bind to their respective cytosolic immunophilins: cyclosporine to cyclophilin and tacrolimus to the FK506-binding proteins FKBP-12 and FKBP-52, a component of the glucocorticoid receptor complex. Immunophilins are a family of highly conserved proteins that likely participate in protein folding. The drug-immunophilin complex binds to and inhibits the activity of the enzyme calcineurin, a calcium/calmodulin-dependent protein phosphatase that is expressed in all mammalian tissues. As a result, the complex interrupts the calcium-dependent signal transduction pathway in T-cells. Inhibition of calcineurin by cyclosporin or tacrolimus leads to interference with translocation to the nucleus of various nuclear factors involved in the transcription of cytokine genes, such as the cytosolic subunit of the nuclear factor of activated T-cells (NF-ATc). It also antagonizes the interaction of the transcription factor, cyclic adenosine monophosphatase (cAMP)-response element binding protein (CREB), with its putative deoxyribonucleic acid (DNA) binding site, which in turn inhibits

cAMP-directed transcriptional events. As a result of calcineurin inhibition, the transcription of early T-cell activation genes is suppressed, affecting the production of interleukin-2 (IL-2) and many other cytokines, such as interleukin-3 (IL-3), interferon-γ (INF-γ), and tumor necrosis factor-α (TNF-α). Figure 2.2 illustrates a simplified model of the intracellular mode of action of tacrolimus and cyclosporine.

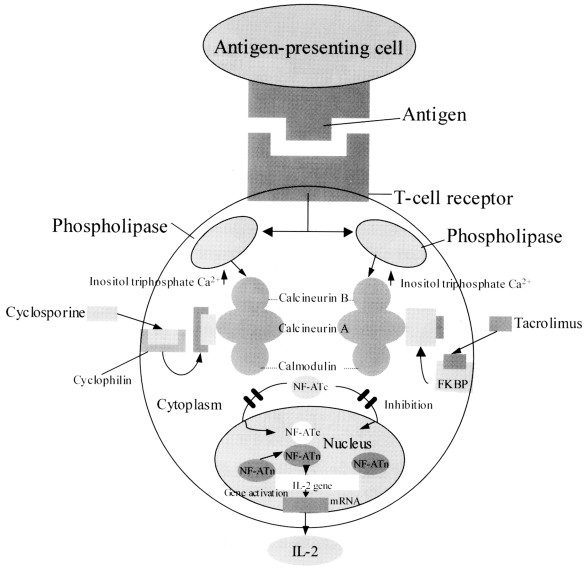

Figure 2.2 Intracellular mode of action of tacrolimus and cyclosporine. Adapted from LC Paul. Mechanistic differences of cornerstone immunosuppressants. ISBN 1-898729-15-8.

2 Absorption, distribution, metabolisation and elimination

2.1 Absorption

Tacrolimus, orally administrated, has a large variability in the rate of absorption and absolute bioavailability which implies that the mean bioavailability is approximately 25% but can range from 5% to 93% [32,33]. In general oral doses of tacrolimus should be 3 to 4 times higher than intravenous doses to achieve comparable drug exposure after oral and intravenous administration. A reduced bioavailability has been reported in patients awaiting renal transplantation[34], in small bowel recipients with open stomas[35], in African American and non-Caucasian individuals[36-40], in diabetic patients[41,42], and following administration of food with a moderate fat content[43-45]. However, the bioavailability is

similar for paediatric and adult transplant patients[42]. In most patients tacrolimus is absorbed rapidly with peak plasma/blood concentrations obtained in 0.5-1 hour[32] however a lag time of 0 to 2 hours has also been reported in some liver transplant recipients[37] or an extended lag time or secondary peaks[34,46,47].
The shape of the plasma concentration-time profile in some patients (sharp peaks), and higher dose-normalised maximum blood concentration at lower doses is seen in some patients who received different doses, are suggestive of the involvement of a zero order/saturable process in the absorption of tacrolimus[38]. The poor aqueous solubility of tacrolimus and alterations in gut motility in transplant patients may be partially responsible for poor and erratic drug uptake. Presystemic metabolism of tacrolimus by gastrointestinal cytochrome P450 (CYP) 3A iso-enzymes and removal by P-glycoprotein transport is extensive[48-50]. P-glycoprotein lowers the intracellular concentration of tacrolimus by pumping absorbed drug back out into the intestinal lumen. P-glycoprotein may also regulate access of tacrolimus to CYP3A enzymes and prevents these enzymes from being overwhelmed by high drug concentrations in the intestine[51,52]. As tacrolimus is repeatedly transported out of the intestinal mucosa cells and then passively reabsorbed, this continous repeated exposure should lead to more efficient metabolism[52,53]. P-glycoprotein belongs to a family of adenosine triphosphate-binding cassette transporters with considerable overlap in substrate specificity. Bile is not essential for tacrolimus absorption[54-56]. Simultaneous administration of enteral feeds does not appear to interfere with tacrolimus bioavailability. Contrasting studies appeared regarding the role of ABCB1 polymorphisms in the tacrolimus metabolisation process. A number of studies[57-65] found a significant association between the ABCB1 polymorphism C3435T or the ABCB1 haplotype consisting of the polymorphisms C1236T, G2677T/A and C3435T and tacrolimus C_0 concentrations or pharmacokinetic parameters, while other studies[66-76] found no significant correlation between these parameters. A large diversity of transplant recipient populations were used to examine this ABCB1 genotype tacrolimus blood concentration correlation which may explain the difference in the results observed. Especially, when the role of these ABCB1 polymorphisms is minor compared to the CYP3A polymorphisms.

2.2 Distribution

Transplant recipients have significantly higher blood tacrolimus concentrations (mean 15 times; range 4 to 114 times) than the corresponding plasma concentrations due to the extensive binding of tacrolimus to the red blood cells[46,54,77-81]. Venkataramanan *et al.*[54] reported that the maximum amount of tacrolimus bound to red blood cells (B_{max}) is 418 $\pm$ 258 μg/l with an apparent dissociation constant (K_D) of 3.8 $\pm$ 4.7 μg/l in transplant patients while B_{max} is 1127 μg/l and K_D of 13.5 μg/l in healthy volunteers. The diffusion of tacrolimus from erythocytes is slow in comparison with the transit time of blood through an organ, but tacrolimus is readily released from the erythrocytes[82-84], and the binding of tacrolimus to erythrocytes may in part protect it from hepatic metabolism[85]. An

intracellular protein in erythrocytes, with a molecular weight range (14 to 15 kDa) corresponding to FKBP[82], or a molecular weight of 11 to 12 kDa[86] appears to be primarily responsible for binding tacrolimus. Tacrolimus does not bind to haemoglobin. When the tacrolimus concentration in whole blood increases, the uptake of tacrolimus by erythrocytes is saturated, resulting in a lower blood *versus* plasma ratio[81,82,84,86]. Approximately 99% of tacrolimus in plasma is bound, with uptake not saturable at physiological drug concentrations[87-89]. Additionally, tacrolimus is principally associated with α_1-acid glycoprotein, lipoproteins, globulins and albumin. Partitioning of tacrolimus between erythrocytes and plasma *ex vivo* is dependent on the haematocrit, tacrolimus concentration, temperature of the sample and plasma protein concentration[87,90-92]. Tacrolimus passes through the placenta and into the breast milk[78,93,94]. In one study[94], 21 female liver transplant recipients treated with tacrolimus before and throughout gestation, the mean tacrolimus concentrations (μg/l) on the day of delivery were 4.3 in the placenta *versus* 1.5, 0.7 and 0.5 in the maternal, cord and child plasma, and 0.6 in the first breast milk specimens. In paediatric liver recipients, a volume of distribution up to 1.8 times higher than in adult recipients has been reported[95]. Possible reasons for this include increased membrane permeability of red blood cells and reduced amounts and affinity of plasma binding proteins in infants, enhancing drug entry into some compartments[96].

2.3 Metabolism

Tacrolimus undergoes O-demethylation, hydroxylation and/or oxidative metabolic reactions, predominantly by CYP3A4 and CYP3A5 in the liver and intestinal wall, with <0.5% of the parent drug appearing unchanged in the urine or faeces[88,90,93,97-108]. Several metabolites are the product of a two-step reaction: oxidation by cytochrome P450 3A enzymes destabilising the macrolide ring and followed by its rearrangement[92,102,105,109]. Schüler *et al.*[109] detected seven different isomers of 13-O-demethyltacrolimus by using two-dimensional homo- and heteronuclear magnetic resonance experiments. Figure 2.3 illustrates the major metabolic pathways of tacrolimus. Several other metabolites have been isolated, the structures of which have only partially been identified using mass spectrometry[32]. The CYP3A subfamily composed of four genes, CYP3A4, CYP3A5, CYP3A7, and CYP3A43, and three pseudogenes CYP3AP1, CYP3AP2 and CYP3AP3, consists of 231 kb and is located on chromosome 7q21-q22.1 [56]. The first human CYP3A to be identified was CYP3A4, which was independently cloned in two separate laboratories in 1986 [110,111]. Another CYP3A member CYP3A5 was subsequently purified from liver[112] and the corresponding complementary DNA (cDNA) was cloned in two separate laboratories[113,114]. A third CYP3A family member, CYP3A7, which is expressed at high levels in fetal liver, was also identified[112,115] and cloned by Komori *et al.*[116]. More recently cDNA clones for a fourth CYP3A member, CYP3A43, have been isolated from the liver[117-119].

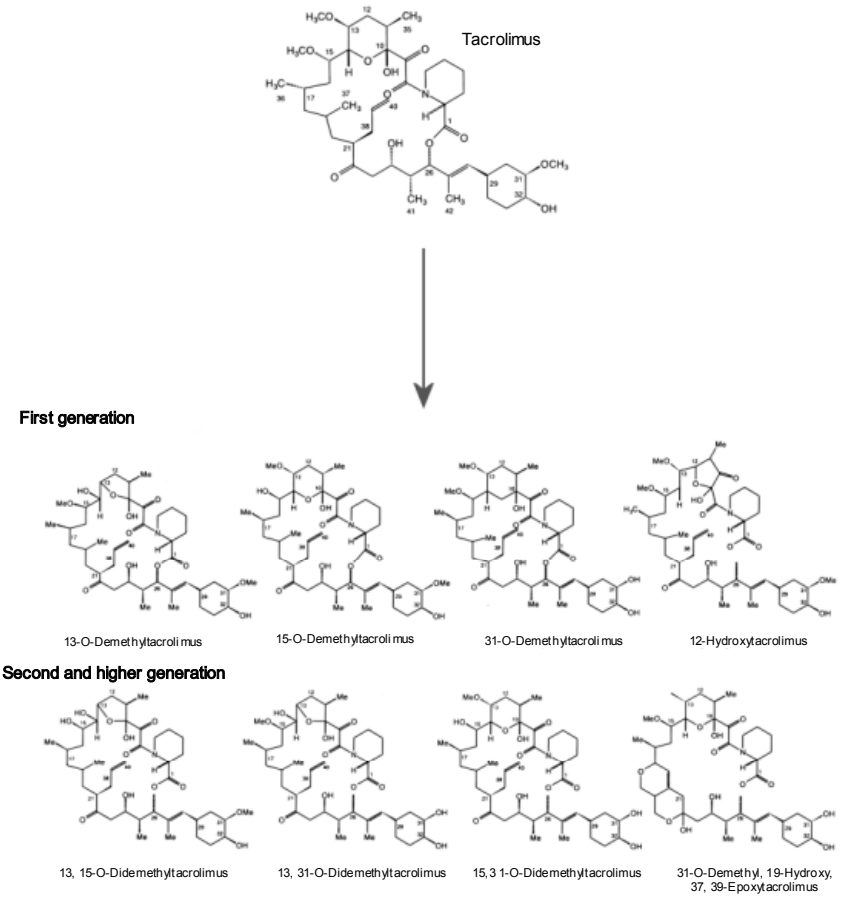

Figure 2.3 The reactions involved in the tacrolimus metabolism are cytochrome P450 3A-mediated hydroxylation, demethylation and oxidation. The proposed metabolite structures and metabolic pathways are based on Iwasaki *et al.*[87,92]. The metabolites are classified as first and second generation metabolites. First generation metabolites are those directly derived from tacrolimus and are changed in one position. Metabolic changes in certain positions such as 13-O-demethyltacrolimus, lead to secondary non-enzymatic rearrangement of the macrolide ring resulting in several isomers. This figure is derived from an illustration of a publication of Christians *et al.*[288] .

2.4 Elimination

The metabolites of tacrolimus are for more than 95% eliminated by the biliary route[97]. Urinary excretion accounts for, on average, 2.4% of tacrolimus elimination. Biliary obstruction is reported to increase the concentration of tacrolimus metabolites in the blood[120,121].

3 Pharmacokinetics

3.1 Genetic factors affecting the pharmacokinetics

3.1.1 Cytochrome P450 (CYP450) 3A4

The CYP3A4 gene is encoded by a 27 kb sequence on chromosome 7q21.3-q22.1 and spans 13 exons[117,122,123]. CYP3A4 consists of 502 amino acids with a molecular weight of 57 kDa[124]. CYP3A4 accounts for approximately 30% of the total cytochrome P450 activity in the liver and 70% of the cytochrome P450 activity in the small intestines[125,126]. Since so much of the human cytochrome P450 activity is due to CYP3A4, it comes as no surprise that CYP3A4 performs the bulk of oxidative drug metabolism in humans. Therefore, the list of medicines that are metabolized by CYP3A4 is still growing and CYP3A4 may be involved in the metabolism of about 50% of all clinically used drugs. CYP3A4 is responsible for the hydroxylation, demethylation, and dealkylation of several endogenous and exogenous compounds. Due to the multiple enzymatic sites on the CYP3A4 molecule, CYP3A4 is able to affect so many compounds through multiple enzymatic steps. CYP3A4 is also an important enzyme in the metabolism of endogenous steroids, cholesterol and lipids through hydroxylation. Children and young adults seem to have more CYP3A4 function than do adults and this enzyme may be more active in women than in men, but studies of age and gender differences with regard to CYP3A4 have yielded conflicting results[127]. CYP3A4 has a vast amount of metabolic activity, and it is often thought of as a high capacity/low affinity enzyme as compared with the other cytochrome P450 iso-enzymes, particularly CYP2D6, CYP2C9 and CYP2C19 which are low capacity/high affinity enzymes. CYP3A4 may function as "sink" where many drugs go to be metabolised at relatively high levels. At low levels, the low capacity/high affinity enzymes perform more of the enzymatic activity. If CYP3A4 is inhibited, the drugs may spill over to the other enzymes, but because these enzymes are low capacity, serum levels of the drugs may increase significantly and lead to toxicity[128]. Drugs with evidence of effective competitive or non-competitive CYP3A4 inhibition include the azole anti-fungals itraconazole[129] and ketoconazole[130], diltiazem[131], the macrolide antibiotics clarithromycin, erythromycin[132], and troleandomycin[133], norfluoxetine, nefazodone[129], the quinolone antibiotics ciprofloxacin and norfloxacin[134], quinupristin/dalfopristin[135], and the protease inhibitors indinavir and ritonavir[136]. Ketoconazole and ritonavir seem to be the most potent of all CYP3A4 inhibitors. A moderate CYP3A4 inhibitor is a chemical (or chemicals) in grapefruit and grapefruit juice[137,138]. The action of this chemical (or these chemicals) is probably through inhibition of the gut wall CYP3A4, although there is some evidence that the effect may be due to P-glycoprotein[139]. However, regardless of the mechanism, when a second drug is ingested after grapefruit juice has been drunk, serum levels of the second drug increase if it is a substrate of CYP3A4 or P-glycoprotein. The strongest known inducer of CYP3A4 is carbamazepine and its mechanism is described by Levy *et*

al.[140]. Carbamazepine induces CYP3A4 and the drug is also metabolised by CYP3A4 however, carbamazepine induces not only CYP3A4 but also phase II conjugation enzymes[141]. Other anti-epileptics such as phenytoin, phenobarbital and primidone[142] also induce CYP3A4. Additionally, there is evidence that rifampicin and rifabutin[143], nevirapine and efavirenz[144,145], troglitazone[146], dexamethasone and prednisone[147] all induce CYP3A4. In addition, St. John's wort is a popular herbal product used to treat depressions and thought to be implicated in drug interactions involving CYP3A4[148,149] and/or P-glycoprotein[150]. Markowitz *et al*.[151] found that a 14-day course of St. John's wort administration significantly induced the activity of CYP3A4 as measured by changes in the pharmacokinetics of alprazolam. This suggested that long-term administration of St. John's wort may result in diminished clinical effectiveness or increased dosage requirement for all CYP3A4 substrates. Use of St John's wort has led to decreased cyclosporine levels, ultimately causing transplantation rejection[152-154]. St John's wort has also been shown to decrease digoxin levels[155]. Given these reports, St John's wort may affect numerous other drugs and make them less effective. Unlike other cytochrome P450 iso-enzymes CYP3A4 is capable of being stimulated. Stimulation is similar to induction in that enhanced CYP3A4 activity occurs. It differs since it happens immediately while induction is delayed by 1 to 3 weeks. Stimulation is termed homotropic if performed by a substrate of CYP3A4 and heterotropic if performed by other effectors or drugs[156]. Although more than 30 CYP3A4 variants have been reported[157], most CYP3A4 variants are SNPs with low allelic frequencies and many of these SNPs are population specific[157]. In addition, the clinical implementation of all CYP3A4 polymorphisms of in drug metabolism have not been determined[119]. Rebbeck *et al*. described the most common variant in the 5'-untranslated region (UTR) of CYP3A4, CYP3A4*1B (A-392G)[158]. The frequency of CYP3A4*1B is highly variable in different racial populations with an allele frequency of 0% in Chinese, Taiwanese and Japanse[159-162], 4-10% (Caucasians)[163-166], 9-10% (Hispanics)[161,162] and 48-80% (African-Americans)[161,162,165,167,168].

3.1.2 Cytochrome P450 (CYP450) 3A5

The CYP3A5 gene has 13 exons encoding 502 amino acids[113,169]. CYP3A5 has been reported to be expressed at higher levels than CYP3A4 in extra hepatic tissue, such as in the small intestine[170], colon[171], lung[172], oesophagus[173], kidney[174,175], adrenal gland[176], anterior pituitary[177], breast[178], prostate[179] and polymorphonuclear leukocytes[180]. The majority of compounds that are substrates for CYP3A4 are also metabolized by CYP3A5, usually with a higher catalytic efficiency. Rendic provided a detailed list of CYP3A4 and CYP3A5 substrates[181]. The induction mechanism for CYP3A4 and CYP3A5 is similar and it is therefore unlikely that drug interactions involving enzyme inducers such as rifampicin will be affected by the CYP3A5 genotype. However, the interactions involving CYP3A enzyme inhibition are probably more common than those involving induction. There is increasing evidence that CYP3A4 and CYP3A5 show differences in their response to enzyme inhibitors and it is therefore possible that

CYP3A5 expression may be a determinant of individual susceptibility to drug interactions involving inhibition. In a detailed study regarding the differences between CYP3A4 and CYP3A5 with respect to inhibition, differences in the extent of inhibition by ketoconazole and diltiazem were confirmed and there was also less inhibition of CYP3A5 than CYP3A4 by erythromycin and nicardipine[182]. Regarding the mechanism-based inhibitors, it was suggested that the lower level of inhibition of CYP3A5 was due to a decreased ability to form the metabolites associated with metabolite-intermediate complex rather than an inability to form such a complex. The CYP3A5*3 variant allele designated for the presence of a genomic A6986G transition within intron 3 is the most common and functionally important variant across all ethnic populations studied[183], while additional rare polymorphisms that affect CYP3A5 activity in individuals expressing the enzyme have also been identified[184]. The non-coding CYP3A5 A6986G polymorphism creates a cryptic consensus splice site in the pre-mRNA, resulting in the incorporation of 131 bp of intron 3 sequence (named exon 3B and inserted between exon 3 and exon 4) in the mature mRNA, and the production of improperly spliced mRNA containing exon 3B (splice variant 1) and a small amount of properly spliced mRNA (wt-mRNA)[157,185]. The insertion causes a frameshift and a predicted premature termination codon, so that the encoded protein is truncated at amino acid residue 102 with loss of enzyme activity. According to the cytochrome P450 nomenclature (see website cytochrome P450 iso-enzyme nomenclature: http://www.cypalleles.ki.se/) any allele positive for 6986A is designated CYP3A5*1 while any allele positive for 6986G is designated CYP3A5*3. The CYP3A5*1 allele has a frequency of 5-7% in European Caucasians compared with approximately 40% among Africans and African Americans, and 25% in various Asian ethnic groups[163,184-193]. Additionally, Xie *et al.*[183] performed a meta-analysis on the ethnic distribution of CYP3A5 alleles and genotypes.

A large number of studies demonstrated the influence of the CYP3A5 genotype on the dose requirement and clearance of the widely used immunosuppressants tacrolimus[58,60,61,64-67,69-71,74-76,194-205], cyclosporine[42,66,67,203,206-213] and sirolimus[74,214-216] in organ transplant patients. A number of studies found the CYP3A5*3 polymorphism a good predictor of the tacrolimus dose requirement and the tacrolimus plasma levels[58,60,61,64-67,69-71,74-76,194-203,205]. Additionally, two studies[67,201] determined using stepwise multiple regression analysis that the CYP3A5 genotype can explain approximately 35% of the variation observed in the tacrolimus concentrations of the transplant patients. The influence of CYP3A5 genotypes on the cyclosporin metabolism has also been examined in a number of studies[66,67,203,206-213,217]. Most of these studies[66,203,206-208] found no significantly increased rate of drug clearance in patients carrying a CYP3A5*1 allele, although some other studies observed a significant decrease in dose adjusted plasma trough levels[67,210-213,217]. Although the reason is not clear why the CYP3A5 genotype is less predictive for cyclosporin than for tacrolimus pharmacokinetics a few possible explanations can be given. The most evident explanation is that tacrolimus might be a better substrate for CY3A5 compared to CYP3A4 than is cyclosporin. Kamden *et al.*[218] demonstrated that the turnover

percentage for CYP3A5 with tacrolimus is approximately 60% higher than that for CYP3A4. This could explain the apparent higher requirement for tacrolimus in patients carrying a CYP3A5*1 allele. The published data on the cyclosporin metabolism is limited and is based on the CYP3A isoforms expressed in HepG2 cells using a vaccinia virus system[113]. In this study the ability of CYP3A5 to form the M17 metabolite was approximately 60% of that of CYP3A4, and no formation of the other oxidation metabolites M1 and M21 could be detected by CYP3A5. Another *in vitro* study[219] demonstrated that CYP3A5 may contribute to the formation of primary and secondary metabolites of cyclosporine, particularly in kidneys carrying the wild type CYP3A5*1 allele. Another possible explanation for the discrepancy between the effect of CYP3A5 genotype on the metabolism of the two immunosuppressants could be related to differences in the extent of the first-pass metabolism. Despite a twofold difference observed in a recent study[201] between patients homozygous for the CYP3A5*1 allele and those homozygous for CYP3A5*3 variant allele, other factors like gender, age, body mass index (BMI), time since transplantation and the use of steroids also may contribute to the wide intersubject variability in dose requirement[67,201,220,221]. Although at the moment the clinical relevance of the association between tacrolimus pharmacokinetics and CYP3A5 remains unclear, it is possible that CYP3A5 genotyping could form part of a genetic test to predict tacrolimus dose requirement. A recent study[202] proposed after examining 19 volunteers between two dialysis periods a twofold higher tacrolimus dose requirement for patients carrying at least one CYP3A5*1 compared to patients carrying a CYP3A5*3 variant allele.

3.1.3 Cytochrome P450 (CYP450) 3A7

The CYP3A7 gene is encoded by a 17.1 kb sequence on the human chromosome 7q22.1 and spans 13 exons[222]. CYP3A7 accounts for between 30 and 50% of the total cytochrome P450 activity in fetal liver[223], although it is now apparent that it is also expressed in adult liver. In adult livers expressing high levels of CYP3A7, it has been suggested that this isoform may contribute up to 20% of the total CYP3A[176]. CYP3A7 is also expressed in adult extra-hepatic tissues, including intestine, endometrium, adrenal gland and prostate[176,224,225]. In the endometrium, expression of CYP3A7 has been demonstrated to occur at high levels during the proliferative phase of the menstrual cycle[226]. Expression patterns in adult intestine seem to parallel those in adult liver[224] but it is still unclear whether expression in other tissues is subject to the same inter-individual variability. The majority of the CYP3A7 substrates are similar to those for CYP3A4 and CYP3A5 except that the V_{max} values tend to be lower and K_m higher in most cases. However, CYP3A7 shows a higher catalytic activity for the retinoic acid isomers all-*trans* retinoic acid, 13-*cis* retinoic acid and 9-*cis* retinoic acid compared with both CYP3A4 and CYP3A5 [227,228]. This may indicate a role for CYP3A7 in certain key developmental processes in view of the central role of retinoic acid in regulating some pathways and the need to maintain very specific local concentrations of this compound during the development[229]. In addition, CYP3A7 shows a high activity in the 16α-

hydroxylation of estrone and dehydroepiandrosterone[115,184]. This may be of considerable relevance to both normal physiological processes and in relation to cancer susceptibility in view of the fact that CYP3A7 has now been shown to be expressed in several different steroid-responsive adult tissues. A study on the induction of CYP3A7 in fetal hepatocytes found induction by dexamethasone but not by rifampicin and suggested that induction was not mediated by the pregnane X receptor (PXR) but by the glucocorticoid receptor[230]. The CYP3A7*1C variant allele was reported at a frequency of 3% in Caucasians, 6% in African American and 0% in Chinese[185,224]; Op den Buijsch *et al*. Submitted 2007. While the CYP3A7*1B and CYP3A7*1D variant alleles have allele frequencies of 1% in Caucasians and the CYP3A7*1E variant allele has been determined in African Americans at a frequency of 8%[185,224]. The cause of CYP3A7 expression in the adult liver appears to be the G291T substitution which results in the creation of a binding site for the hepatocyte transcription factor hepatocyte nuclear factor-3 (HNF-3), whereas the A232C change destroys an HNF-3 binding site and creates a putative octamer motif identical to that found in the upstream sequence of CYP3A4 [231].

3.1.4 Uridine 5'-diphosphate glucuronosyltransferase (UGT) 2B7

The analysis of tacrolimus metabolites in humans has provided evidence not only for CYP3A catalysed metabolism, but also for the formation of glucuronides[232]. Strassburg *et al*.[233] examined the glucuronidation activities of different uridine 5'-diphosphate glucuronosyltransferase (UGT) subfamilies for both tacrolimus and cyclosporine and found that UGT2B7 showed the highest glucuronidation activity in the human gastrointestinal tract. The UGT family, a group of proteins responsible for the glucuronidation of several endogene and exogene compounds in humans, is mainly present in the liver. However, UGTs are also found throughout the gastrointestinal tract, where they are an integral part of prehepatic first-pass metabolism. In addition, UGTs also work in the kidneys, brain, placenta and in several other locations in the human body. The major function of glucuronidation is to increase the polarity of the target compounds, a process which facilitates their detoxification and excretion. However, glucuronidation can also result in compounds which are biologically active or demonstrate increased toxicity. After glucuronidation, intestinal bacterial β-glucuronidases break down glucuronidation products and release the unconjugated drugs via enterohepatic recirculation. This "recycling" system slowly clears conjugated compounds and releases glucuronides for re-use[234-238]. Metabolites of tacrolimus have been isolated from human plasma, bile and urine and have been generated in human liver microsomes[98,120,239,240]. Up to 15 metabolites (demethyl-, demethylhydroxy-, didemethyl-, didemethylhydroxy-, and hydroxytacrolimus) have been identified in blood samples obtained from liver and renal transplant recipients, with the demethyl (approximately 3% of the area under the concentrationtime curve (AUC) of tacrolimus) and the demethylhydroxy (approximately 10% of the AUC of tacrolimus) compounds being the most prevalent[121]. Tacrolimus is predominantly metabolised into 13-O-

demethyltacrolimus in liver and blood[98,120,239,240]. This metabolite was found to be approximately one-tenth as active as tacrolimus[87,92]. Based on the blood concentration data, tacrolimus is considered a low clearance drug with clearance equivalent to 3% of the liver blood flow[97]. Figure 2.4 illustrates an overview of the tacrolimus metabolism.

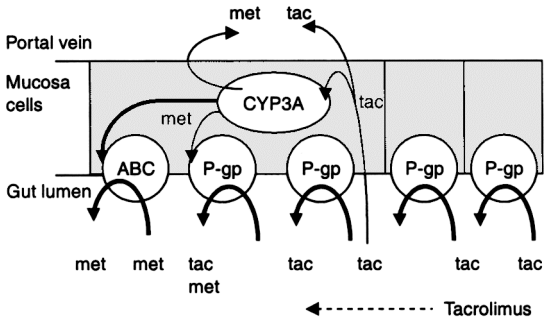

Figure 2.4 Proposed interactions between tacrolimus metabolism and active efflux in the small intestine mucosa. Two potential cooperative mechanisms between cytochrome P450 (CYP) enzymes and active efflux transporters have been proposed. P-glycoprotein regulates the access of drugs to CYP enzymes and prevents CYP enzymes from being overwhelmed by the high drug concentrations in the intestine[51,52,369]. With the drug being repeatedly transported out of the mucosa cells and being reabsorbed, repeated exposure leads to more efficient metabolism. The metabolites are better substrates of another active transporter than the parent, thus avoiding inhibition of metabolite efflux by the high concentrations of the parent drug[370]. ABC = ATP binding cassette transporter other than P-glycoprotein; met = tacrolimus metabolite; P-gp = P-glycoprotein; tac = tacrolimus.

3.1.5 Pregnane X receptor (PXR)

The pregnane X receptor (PXR)[241] also known as SXR[242], hPAR[243] or NR (nuclear receptor) 1I2 has been identified as a transcriptional regulator of CYP3A4 [241,243] and ABCB1[244,245]. PXR binds as a heterodimer with the 9-*cis* retinoic acid receptor (RXR; NR2B) to previously characterised xenobiotic response elements in CYP3A gene promoters, and importantly, PXR is activated by the spectrum of chemicals that are known to induce CYP3A gene expression[246,247]. Single nucleotide polymorphisms (SNPs) in the PXR gene can possibly influence the PXR activity and thereby the CYP3A4 and ABCB1 expression.

3.1.6 Constitutive androstane receptor (CAR)

The consititutive androstane receptor (CAR) encoded by NR1I3 is a member of the orphan nuclear receptor superfamily that plays a major role in the control of drug metabolism and disposition[248]. CAR binds to the DNA of target genes as a heterodimer with the retinoid X receptor (RXRα) and regulates gene transcription[249-251]. CAR is predominantly expressed in the liver and intestine[252,253]. CAR regulates transcription of

the genes encoding drug/steroid metabolizing enzymes as well as other physiologically important enzymes. Moreover, CAR transactivates several hepatic cytochrome P450s and other enzymes involved in drug metabolism including UGT1A1 [254], CYP2B6 [255], CYP3A4 [251], CYP2C9 [251,255] and CYP2C19 [256]. Therefore, polymorphisms inducing changes in the function or expression of the NR1I3 are thought to be potential sources for variation in cytochrome P450 expression or drug metabolism in humans.

3.1.7 Glucocorticoid receptor (GR)

The glucocorticoid receptor (GR), encoded by NR3C1, is a member of the nuclear hormone receptor of transcription factors. In the cytosol, GRs are associated with heat-shock and other proteins, and the binding of glucocorticoid leads to their nuclear translocation and positive or negative regulation of various genes[257,258]. The GR causes anti-inflammatory effects through transcriptional activation of glucocorticoid induced leucine zipper genes or transcriptional suppression of genes of inflammatory cytokines induced by NF-kappaB or AP-1 [259-261]. Therefore, it is possible that the altered transcriptional activity of GR associated with polymorphisms of NR3C1 might affect the expression levels of target genes including CYP450 enzymes. Furthermore the GR regulates the expression of many drug metabolising enzymes. For instance, it is reported that GR activates the transcription of drug metabolizing enzymes CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP3A4 and CYP3A5 through GREs in the promoter/enhancer regions of these genes or induction of both pregnane X receptor and constitutive androstane receptor[251,256,262-265]. Individual differences of CYP450 enzymes are thought to result from differences in their expression levels and/or activities[265].

3.2 Non-genetic factors affecting the pharmacokinetics

3.2.1 Sex

Fitzsimmons *et al.*[36] observed no sex-specific effects on the pharmacokinetic profile of tacrolimus after examining the pharmacokinetic data collected from 127 adult (49 female and 78 male) who participated in three phase I studies and five multicentre, phase II clinical trails and 343 transplant recipients (149 female and 194 male) who participated in two phase III clinical trails. Healthy volunteers, pre and posttransplant renal recipients and bone marrow recipients demonstrated no sex differences in pharmacokinetics. However, Kuypers *et al.*[220] recently reported that female renal transplant recipients reached overall higher C_{max} levels compared to their male counterparts (mean 26.1 ± 9.1 μg/l *versus* 22.5 ± 8.8 μg/l; $P = 0.04$, respectively) and six months after grafting, the tacrolimus $AUC_{0\text{-}12}$ was significantly higher in female renal transplant recipients (148.7 ± 35.2 μg × hr/l *versus* 132.9 ± 32.5 μg × hr/l; $P = 0.003$)

3.2.2 Age

Numerous studies already demonstrated that paediatric transplant recipients require two to fourfold higher tacrolimus doses than adults to maintain similar trough concentrations[95,266-267]. The higher tacrolimus doses required in paediatric patients have been attributed to differences in cytochrome P450 3A although differences in bowel length, hepatic blood flow and P-glycoprotein expression also need to be considered[272]. The CYP3A4 content in hepatic tissue is extremely low in the foetus but increases rapidly after birth to 120% of adult levels after the age of one year. In addition, CYP3A5 is expressed in nearly 50% of all infant livers, but could be found in only 29% of adult livers[273]. However, it is unclear whether intestinal CYP3A expression changes in parallel with hepatic CYP3A expression during human maturation. Moreover, it is also not known whether P-glycoprotein expression changes with age. In addition Kuypers *et al.*[220] found that six months after grafting the tacrolimus AUC_{0-12} increased with younger age (R^2 = -0.20; P = 0.007). Tacrolimus trough levels were lower with increasing age (R^2 = -0.13; P = 0.05 day 7 and R^2 = -0.20; P = 0.005 at six months).

3.2.3 Race

African-American transplant patients require higher tacrolimus dosages (mg/kg) than Asians (Chinese or Japanese) and Caucasians[36-40,274,275]. Moreover, bioavailability was significantly reduced among Black patients (9.9% *versus* 19%)[36]. After oral tacrolimus administration, tacrolimus C_{max} were significantly lower in the African American than in the White transplant patients. The differences between ethnic groups may result from racial differences in intestinal CYP3A or P-glycoprotein activity[40,275].

3.2.4 Haematocrit and albumin concentrations

Undre *et al.*[276] found, after examining 303 renal transplant recipients, a correlation between the relative clearance and both haematocrit (r = 0.81) and albumin (r = 0.74) concentration over the first 12 weeks posttransplantation. After examining blood samples from 33 patients with autoimmune diseases and recipients of bone marrow and liver transplant undergoing cyclosporine immunosuppressive therapy, Kuzuya *et al.*[277] found, after adding tacrolimus to each blood sample at a final concentration of 7.5 ug/l and 15 ug/l, that haematocrit interferes with the MEIA II for tacrolimus. Furthermore, the magnitude of the interference is clinically significant, especially during the early posttransplantation period, because the haematocrit fluctuates widely and the target concentration of tacrolimus is variable. Additionally, Kuzuya *et al.*[277] even proposed that beyond the range from 30% to 40% of haematocrit values, caution should be exercised in interpreting results as one may need to compensate for the levels of tacrolimus.
Recently Brown *et al.*[278] demonstrated, after examining 1156 blood samples obtained from 121 paediatric recipients of renal, liver and bone marrow grafts or hepatocyte or pancreatic islet cell implants, that patients with a low packed cell volume and plasma albumin are likely to show artificially high tacrolimus concentration when measured by

MEIA. This increased risk of underimmunosuppression must be considered should doses be reduced to lower these seemingly high tacrolimus concentrations. More recently Cheung *et al.*[201] also reported, after using stepwise multiple regression analysis, that the haematocrit levels may explain 3,5% of the variability in the tacrolimus blood concentrations. Knowing that in this study Cheung *et al.*[201] used blood samples obtained from a wide spread period after transplantation namely (133-4982 days) it is likely that this percentage will be much higher in a transplant recipient group just after transplantation. These results are in line with the strong binding of tacrolimus to red blood cells and serum albumin. Haematocrit and α_1-acid glycoprotein concentrations are generally lower in renal transplant patients immediately post surgery and increase significantly when the patients recovers[279]. Ghoshal *et al.*[280] demonstrated a correlation between IMx II and LC MS/MS of respectively r = 0.612 and 0.829 for tacrolimus concentrations above and below 9 ng/ml which may imply that influence of a low haematocrit fraction is minor for tacrolimus concentration above 9 ng/ml. Additionally, Ghosal *et al.*[280] suggested that tacrolimus concentrations below 9 ng/ml measured by MEIA II are questionable and should be interpreted with caution.

3.2.5 Time after transplant action

Several studies among patients who underwent a different organ transplantation have reported a decrease in the dosage of tacrolimus required to maintain similar trough concentrations with increasing time posttransplant[42,276,281,282]. A generally considered cause for the decrease in the tacrolimus dosage is a decrease in the tacrolimus clearance with time, although an increased bioavailability also has to be considered as a feasible explanation. Pou *et al.*[281] found, in 50 adult liver transplant recipients, significant reductions in the dose-normalised trough concentration of tacrolimus after the first and third month of treatment. Additionally, Undre *et al.*[276] demonstrated in 303 renal transplant recipients after two years that the decline in the dose-normalised trough concentration was 61.1% and the tacrolimus dosage was reduced by 50%. Potential explanations for this are a reduction in the corticosteroid dosage and the increased haematocrit and albumin concentrations.

3.2.6 Corticosteroid dosage

The concomitant use of corticosteroids, which may induce CYP3A iso-enzymes, also has the potential to influence the tacrolimus elimination although contradictory studies appeared. Undre *et al.*[276] found, after examining 303 renal transplant recipients, a significant correlation between the relative clearance and the mean oral corticosteroid dosage (r = 0.94) during months 2-12 posttransplant which indicates that corticosteroids increase the tacrolimus metabolism. Other studies demonstrated, or at least suggested the opposite effect[283,284], while researchers examining bone marrow transplant patients could not demonstrate any influence[285].

3.2.7 Population

A higher tacrolimus clearance was found for adult renal transplant recipients compared to liver transplant recipients and healthy volunteers[286,287]. The presence of low haematocrit and albumin concentrations and differences in corticosteroid dosage may be partly responsible for this observations[287,288]. A 38% reduction in the AUC_{0-12} associated with tacrolimus was reported in diabetics compared to non-diabetic patients pre-transplant[41]. This may be due to disturbed gastrointestinal motility in patients with diabetes mellitus.

3.2.8 Hepatic dysfunction

Several studies already reported that a poor liver function can decrease tacrolimus clearance up to 67% and increase the elimination half-life with a threefold[283,286,289-292]. Cold ischaemia time and reperfusion injury to a transplanted liver may also alter the clearance of tacrolimus. However, tacrolimus clearance has been reported to be similar between healthy volunteers and patients with mild hepatic impairment[289]. Transplant patient who are hepatitis C positive require a significantly lower mean dosage of tacrolimus than hepatic C negative patients to obtain the same trough concentrations[268,293,294]. Horina *et al.*[295] suggested that replication of the hepatitis virus in liver cells alters the cytochrome P450 system which results in reduced tacrolimus metabolism.

3.2.9 Renal function

No significant correlations have been reported between serum creatinine concentrations and the clearance of tacrolimus ($r = 0.36$)[34]. Patients with severe renal dysfunction (serum creatinine concentrations of 344 - 1061 μmol/l) and patients on dialysis prior to renal transplantation have similar tacrolimus clearance to healthy volunteers.

3.2.10 Donor liver characteristics

The characteristics of the donor organ may influence the pharmacokinetics of tacrolimus in liver transplant recipients[296]. It is demonstrated in 118 liver transplant recipients that the mean dosage requirements were lower in patients who received a liver from donors of an older age group (≥ 65 years) than from donors of a younger age group (10-25 years), possibly as a result of decreased metabolic capacity in older livers. Additionally, several studies have reported that transplant patients who received a partial liver from a living donor require significantly lower tacrolimus doses than transplant patients who received a whole liver from a deceased donor to achieve similar tacrolimus trough concentrations in the early posttransplant period[297-299]. Two studies performed in transplant patients of related living-donor liver transplant (LDLT), the tacrolimus dose needed to reach a target blood concentration correlated with the ratio of graft weight to

recipient standard liver volume which indicating an increased metabolic capacity with increase graft size[300,301].

3.2.11 Impact of organ donor genotype

CYP3A is expressed in the enterocyte and the liver, while the P-gp barrier to drug absorption probably only acts at the levels of the intestine. Liver transplantation offers a fascinating opportunity to study the impact of different genotypes in the intestine and liver with metabolism of drugs by the transplantated liver. A further layer of complexity is added by the functional regeneration of hepatic grafts with time which may lead to a progressively increasing role in drug metabolism over time[302,303].

3.2.12 Administration of food

The influence of food on the oral absorption of tacrolimus appears to be dependent on its fat content and relative time of administration. Previous research indicated that coadministration of low fat food had minimal effect on the extent of absorption, but delayed the time to reach C_{max}[304]. A significant reduction in both the rate and extent of tacrolimus absorption (bioavailability reduced 27%) was observed after concurrent administration of moderate fat food[43]. Another study examined the effect of meal-timing on the tacrolimus absorption and found that the tacrolimus absorption in the fasting state had a significantly greater relative bioavailability than all other treatments[44]. Moreover, the absorption of tacrolimus was also significantly prolonged after a meal. Van Duijnhoven *et al.*[305] recently examined in 27 renal transplant recipients that conversion to non-fasting ingestion of tacrolimus, without dosage adjustments did not significantly change the tacrolimus trough concentrations in stable patients.

4 Drug-drug interactions

After oral administration, there are several factors involved in absorption of a drug which all can be the target of drug interactions: delivery to the intestine (pH, gastric emptying and food), absorption from the intestinal lumen (dissolution, lipophilicity, stability active uptake), intestinal metabolism (phase I and phase II metabolism), active intestinal drug efflux pumps, and subsequent hepatic first pass extraction[51,306]. Previously, Christians *et al.*[288] summarises many relevant drug interactions, the information on which the drug interactions are based (*in vitro* / animal studies, controlled clinical trails and clinical observations), their proposed mechanisms and their clinical effects. Recently, Wilkinson[307] described in a review article about the influence of both inducers and inhibitors on felodipine blood levels (a blood pressure lowering drug). Similar reports have also been written for tacrolimus interactions[308-311]. This emphasised that to decrease the variability in the tacrolimus exposure among transplant patients multiple factors like pharmacokinetics (C_0, abbreviated AUC_{0-12} or complete 12 hour AUC),

genetics (CYP3A, ABCB1, UGT2B7 and PXR polymorphisms, the possible influence of co-medication and the influence of other compounds that the patient eventually use like St John's wort or grapefruit juice.

5 Therapeutic drug monitoring

Tacrolimus has a narrow therapeutic index and highly variable pharmacokinetic characteristics. Close monitoring of the tacrolimus concentration is required to achieve an optimal efficiency and thus minimizing the risk of subtherapeutic or toxic blood concentrations. Efficacy and side effects of tacrolimus are highly correlated with the area under the curve (AUC_{0-12})[312]. The most exact way to monitor the total tacrolimus exposure is by creating 12 hour pharmacokinetic profiles, which implicates that the tacrolimus concentration should be measured at at least 6 different time points. The AUC_{0-12} can then be calculated according to the trapezoidal rule using the tacrolimus concentrations measured at different time points. Since recording a complete 12 hour pharmacokinetic profile for every patient is not feasible in clinical practice, traditionally many transplant centres use tacrolimus trough (C_0) concentrations to estimate the tacrolimus exposure. Although tacrolimus C_0 concentrations are generally considered to be a good indication of the total systemic drug exposure[312,313], its usefulness in differentiating graft rejection episodes from nephrotoxicity has been questioned[314-317]. Recently, the correlation between individual tacrolimus concentrations and AUC_{0-12} has been studied in kidney[220,318-323], liver[324], heart[325,326] and lung[327] transplant recipients. In these studies a poor association was found between the tacrolimus C_0 concentrations and the AUC_{0-12} while tacrolimus concentrations measured at other time points showed much better correlations with the AUC_{0-12}. Additionally, strategies have been developed that consisted of a limited number of sampling time points within a short time post dose, the so called limited sampling strategies. Several two and three time point sampling strategies showed a high correlation with the AUC_{0-12} in the published studies and were able to predict the AUC_{0-12} more accurately than the C_0 concentration alone[318,320,321,323,325,327]. Moreover, Op den Buijsch *et al.*[328] recently evaluated 24 different limited sampling strategies for tacrolimus that already have been published in a different renal transplant recipient group. They found that the limited sampling strategies that used two (C_2 and C_4) or three (C_0, C_2 and C_4) sample time points demonstrated the best results in their own renal transplant group. Based on the half-life of tacrolimus which is approximately ten hours, it is necessary to wait at least 36 hours (3.3 half-lives) to reach a steady state tacrolimus concentration after initiation of therapy or after a change in the administration regime of tacrolimus. Ideally, after starting the infusion, blood concentrations should be monitored on day 2 or 3 on average 3 to 7 times weekly during the first few weeks after transplantation, and less frequently thereafter. Special circumstances such as changes in liver function, presence of adverse effects or use of drugs that may alter tacrolimus kinetics may warrant more frequent monitoring[329].

6 Analytical methods used to determine the tacrolimus blood concentration

Tacrolimus concentrations in biological fluids have been measured using a number of methods. However, the development of a simple, specific and sensitive assay method for measuring tacrolimus in biological fluids is limited by the low absorptivity of tacrolimus, the low concentrations of tacrolimus in plasma or blood and the presence of several other drugs in the blood samples of patients, which potentially interfere with the analysis of tacrolimus. The currently available assays can be categorized as enzyme immunoassays, chromatographic/mass spectrometric assays, radioreceptor assay and a bioassay.

6.1 Enzyme immunoassays

Tamura *et al*.[330] reported in 1987 the first method for quantification of tacrolimus in plasma using an enzyme-linked immunosorbent assay (ELISA) method following a solid phase extraction procedure to separate tacrolimus from other components in the sample. The clinical application of this assay was first reported in 1990[55] and a modification of this method has been used to measure tacrolimus in whole blood[77,317]. A number of drugs (Table 2.1) used to treat transplant patients do not appear to cross-react with the antibody used in the ELISA procedure[77,331,332]. A semi-automated technique, based on the principle of microparticle enzyme immunoassay (MEIA) method for the IMx analyser developed by Abbott that measures the concentrations of tacrolimus in whole blood has been reported[333]. The immunoassay MEIA I assay was found to have insufficient sensitivity to monitor patients with tacrolimus concentrations less than 5.0 ng/ml and is also not completely specific to tacrolimus with a cross reactivity of 56.2% and 5.4% for respectively 15-O-demethyltacrolimus and 13-O-demethyltacrolimus[334]. Therefore, the development of a MEIA II method with a suggested lower analytical range (1.5 ng/ml) was released[335].

Table 2.1 Drugs which do not appear to cross-react with the antibody used in enzyme-linked immunosorbent assay (ELISA)[77,87,92,331,332].

Amikacin	Netilmicin
Amphotericin B	Nifedipine
Azathioprine	Paracetamol (acetaminophen)
Carbamazepine	Phenobarbital (phenobarbitone)
Cyclosporin	Phenytoin
Digitoxin	Prednisolone
Digoxin	Primadone
Disopyramide	Procainamide
Erythromycin	Quinidine
Ethosuximide	Salicylates
Flecainide	Theophylline
Fluconazole	Tobramycin
Gentamycin	Valproic acid
Lidocaine (Lignocaine)	Vancomycin
Methylprednisolone	

6.2 Chromatographic / mass spectrometric methods

An LC tandem mass spectrometric (LC MS/MS) method for measuring tacrolimus and its metabolites in patients' blood, bile and urine samples is also available[120,239,240]. This method involves solid-phase extraction of the biological samples and the use of LC to separate various components, followed by the use of a mass spectrometer as a detector. Although the LC MS/MS assay is highly specific due to its capability of quantifying tacrolimus and its major metabolites and its sensitivity with a detection limit of 0.2 ng/ml, the lack of routine availability of this instrumentation in transplant centres, the difficulty in analysing large volumes of samples on a regular basis, and the use of this technique is limited to pharmacokinetic and metabolism studies at the present time.

6.3 Radioreceptor assay

The radioreceptor assay uses tritiated dihydrotacrolimus for competition with tacrolimus extracted from the blood sample to bind partially purified preparation of FK binding protein (FKBP)[336]. The radioreceptor assay is simple to perform, requires a small volume of blood, can provide a rapid turn-around time and the results of this assay correlate well with the whole blood ELISA assay ($r = 0.97$). However, consistently higher tacrolimus concentrations are estimated by this assay in comparison to the ELISA, indicating that the assay is non-specific. It is not clear whether the affinity of a molecule towards FKBP is related to the immunosuppressive activity of that molecule. Any further development of the radio receptor assay dependent on establishing a relationship between the factors mentioned above.

6.4 Bioassay

Zeevi *et al.*[337] reported a biological assay based on inhibition of the allo-antigen-driven proliferation of a clone of allo-reactive T-cells. Although in this bioassay the metabolites with activity are being measured as tacolimus in a biological specimen, the limitations of this procedure are the slow turn around time (> 72 hours) and the inability to directly assay whole blood samples.

7 Comparison of the analytical methods

Venkataramanan *et al.*[32] reviewed several studies that compared two or more analytical methods for measuring tacrolimus. The Protac ELISA, the MEIA and the LC tandem MS method are currently used clinically to measure the blood concentrations of tacrolimus. Whereas the ELISA method generally tends to have a higher coefficient of variation than the MEIA method, the MEIA method lacks the sensitivity required for routine clinical use. The tacrolimus blood concentrations measured by MEIA have been reported to correlate well with those of the ELISA method[317,338,339] since both methods are non-specific as they also measure some of the tacrolimus metabolites. A method which uses LC prior to ELISA, MEIA or a mass spectrometric method is specific for the parent tacrolimus molecule. Other methods seem to measure additional tacrolimus-related components in blood or plasma, owing to the non-specificity of the monoclonal antibody used. Larger discrepancies between different methods are observed in blood samples obtained from patients with an impaired liver function, indicating the accumulation of some metabolites of tacrolimus which cross-react with the antibody used in the assay procedure.

8 Toxicity

Generally occurring adverse effects associated with tacrolimus include nephrotoxicity, neurotoxicity, diabetogenesis, gastrointestinal disturbances, hypertension, infections and malignant complications[3,340,341]. Adverse reactions of tacrolimus tend to occur the most frequently in the first few months after transplantation and decrease with time possibly in line with reductions in tacrolimus concentration[341,342]. At higher tacrolimus concentrations nephrotoxicity, neurotoxicity, diabetogenesis, gastrointestinal disturbances and infections occur more frequently or are more severe[340,341,343-347].

8.1 Nephrotoxicity

The use of tacrolimus can be limited by nephrotoxicity which occurs in approximately 50% of the patients treated with this drug[340,341] although it is not always easy to attribute

renal failure directly to tacrolimus toxicity since transplant patients often also receive other nephrotoxic drugs and may have pre-existing or ongoing kidney diseases (e.g. diabetic patients).

8.2 Neurotoxicity

Minor neurological adverse effects include insomnia, mild tremors, headache, photophobia, nightmares and hyperaethesis[340,341]. Clinical studies point out that tremors occurred in 35 - 56% of the transplant patients receiving tacrolimus while headaches occurred in 20 - 64%, insomnia in 24 - 32% and paraesthesia in 14 - 40%[341,342]. Higher blood concentrations of tacrolimus are associated with less occurring severe neurological events like seizures, akinetic mutism, expressive aphasia, coma and delirium. Liver transplant recipients requiring a transplantation due to hepatitis B or C have an increased risk of severe tacrolimusinduced neurotoxicity[344,348,349].

8.3 Diabetogenic effects

One of the most serious adverse effects of tacrolimus is posttransplant diabetes and this is most likely exacerbated by concomitant administration of corticosteroids[347,350]. Three major randomised, multicentre trails[340,341] demonstrated that the incidence of diabetes in the first year after renal or liver transplantation ranged from 8% to 20%. Bonomini *et al.*[351] reported, after examining 245 renal transplant patients treated with triple therapy (tacrolimus, corticosteroids and azathioprine), an 4% incidence of new onset type I diabetes, while among the 246 patients receiving a dual therapy (tacrolimus and corticosteroids) the incidence of new onset type I diabetes was 5.6% after a follow up period of three months. Posttransplant diabetes is reversible in some patients[340,341]. Risk factors for the development of posttransplant diabetes include, race, high tacrolimus trough concentrations and a high corticosteroid dosage[341].

8.4 Hypertension

Hypertension occurred in up to 50% of the liver and renal transplant patients treated with tacrolimus in several clinical studies[340,341] although it is difficult to determine with certainty an association between tacrolimus therapy and hypertension[352,353]. In transplant patients who were previously normotensive, hypertension may be caused by the excessive intravascular volume, intrinsic renal damage or increased vasomotor tone[352]. Moreover, corticosteroids may also lead to excess water retention, while liver failure prior to transplantation causing low peripheral resistance can mask pre-existing blood pressure problems[353].

8.5 Gastrointestinal disturbances

Several major clinical studies in renal and liver transplant recipients indicated that gastrointestinal disturbances like diarrhoea, nausea and constipation with frequencies of respectively 22 - 72%, 17 - 46% and 31 - 35% were common in patients treated with tacrolimus[340,341].

8.6 Infectious complications and malignancies

It is known that all immunosuppressive drugs increase the risk of infection and malignancy, especially lymphoma. In large clinical studies similar infection rates were found in the tacrolimus and cyclosporine treated groups in renal and liver transplant patients. Within the first year after transplantation approximately 75% of the patients had one or more infections[341,344,354]. According to two studies[341,354] malignancies (excluding lymphoma or lymphoproliferative disease) developed in 1% of the adult renal transplant patients taking tacrolimus and the rate of lymphomas was approximately 1.5%[341,354].

8.7 Other adverse effects

During the tacrolimus therapy mild hyperkalaemia, associated with low or low-normal renin and aldosterone concentrations occur regularly[352,355-357] and is not always correctable by dosage adjustment and may even occur at low blood tacrolimus concentrations[346]. Furthermore, hypomagnesaemia has also been reported during tacrolimus treatment in renal transplant recipients and appears to be related to impairment of normal magnesium conserving mechanisms[358].

8.8 Paediatric patients

The acceptability profile of tacrolimus in children is generally similar to that in adults[3,347,359-361]. However, children appear to be at an increased risk of, to the Epstein-Barr virus related, posttransplant lymphoproliferative disorders[22,347,362-367]. Although some data suggest that paediatric patients may be at a particular risk of developing tacrolimus associated diabetes[368,369], further study is required to confirm these findings.

9 Concluding remarks

Tacrolimus is an immunosuppressive drug with a large inter- and intra-individual variation in its pharmacokinetics, with variable rates and extents of absorption, variable extents of blood protein binding and variable rates of elimination. It is incompletely bioavailable after oral administration, is bound extensively to red blood cells (the binding being saturable), is primarily eliminated by hepatic metabolism and has a narrow therapeutic index. Furthermore, tacrolimus is predominantely metabolised by

cytochrome P450 (CYP450) 3A iso-enzymes into 13-O-demethyltacrolimus which has a neglectable immunosuppressive activity. Previously, several studies have indicated that the CYP3A5 A6986G polymorphism is the most relevant polymorphism present in the CYP450 3A iso-enzyme family with a significant impact on both the tacrolimus exposure and the daily tacrolimus dose. In order to decrease the variability in the tacrolimus exposure of transplant recipients it is also recommendable to look for possible drug interactions and possible interactions with other CYP450 inducers or inhibitors like St John's wort or grapefruit juice.

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