Chapter 12

Influence of pregnane X receptor (PXR) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients

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Abstract

Background and aim

Tacrolimus has a narrow therapeutic index and shows substantial inter-individual variability in its pharmacokinetic characteristics. The pregnane X receptor (PXR) identified as a transcriptional regulator of cytochrome P450 3A4 may play a role in the tacrolimus metabolism. In this study, the role of PXR polymorphisms is examined in an early and a late posttransplant patient group with and without corticosteroids.

Materials and methods

Twenty-six early and thirty-seven late posttransplant patients were genotyped for PXR polymorphisms. The dose-normalised trough concentrations, area under the time concentration curves ($dnAUC_{0\text{-}12}$) and maximum concentrations were determined and correlated with the corresponding PXR genotypes.

Results

Early posttransplant patients carrying PXR variant alleles have significantly lower $dnAUC_{0\text{-}12}$ (1858 *versus* 1243 ng × hr/ml per mg/kg; Mann-Whitney, $P = 0.040$) compared to patients that carry PXR wild type alleles while in the late posttransplant patient group no difference is observed regarding the $dnAUC_{0\text{-}12}$.

Conclusion

Early posttransplant patients carrying PXR variant alleles and using corticosteroids have lower pharmacokinetic parameters compared to patients carrying PXR wild type alleles.

Introduction

Tacrolimus, a member of the calcineurin inhibitor family, has highly variable pharmacokinetic characteristics and a narrow therapeutic index which complicates its daily dose requirement. Achieving optimal exposure is of critical importance especially during the initial period after transplantation, when the risk of rejection or toxicological dosis is the greatest. Therefore, identification of predictive parameters for an optimal tacrolimus dosage would be of great value in clinical practice to determine the best possible therapeutic tacrolimus concentration. The CYP3A iso-enzymes mainly represented by CYP3A4 and CYP3A5 have been identified as the most important enzymes responsible for the tacrolimus metabolism[1]. Although a number of polymorphic CYP3A4 alleles have been identified (http://www.imm.ki.se/CYPalleles/cyp3a4.htm), these are rare and do not contribute significantly to the CYP3A4 expression variability[2]. In contrast to CYP3A4, the expression of CYP3A5 is determined predominantly by polymorphisms[3-9]. Tacrolimus is also a substrate for P-glycoprotein (P-gp), the product of the adenosine triphosphate-binding cassette gene B1 (ABCB1) which is also known as multidrug resistance gene-1 (MDR1)[10]. P-gp is an ATP-dependent efflux pump that contributes to the protection of the body from environmental toxins by limiting their absorption from the gut lumen or increasing their biliairy and urinary excretion and is thus able to modulate the bioavailability of tacrolimus. The nuclear hormone receptors are transcription factors regulating the induction of many genes. Kliewer[11] and Blumberg[12] demonstrated that one member of this family, the pregnane X receptor/steroid and xenobiotic receptor (PXR/SXR) mediates steroid and drug induction of detoxification genes including the drug metabolizing enzyme CYP3A and the drug transporter ABCB1. Since CYP3A4 catalyses the oxidative metabolism of one-half of the clinically administered drugs and ABCB1 effluxes many drugs from human enterocytes and hepatocytes, administration of PXR ligands leads to accelerated clearance of co-administered CYP3A4 and ABCB1 substrates and are thus a major basis for drug interactions. Moreover, there is significant variation among individuals in the extent to which rifampicin can induce hepatic and intestinal CYP3A4 and ABCB1 expression in humans[13]. Single nucleotide polymorphisms (SNPs) in PXR may be a major contributor in the induction variability of CYP3A4 and ABCB1. Zhang *et al.*[13] identified 38 polymorphisms in the PXR gene and found that the 7635A to G transition, and the 8055C to T substitution in respectively intron 5 and 6 of the PXR gene were associated with a higher magnitude of induction of intestinal CYP3A by rifampicin. In addition, the 3'-untranslated (3' UTR) region polymorphisms 11156A to C and 11193T to C showed that those individuals heterozygous with at least one 11156C or one 11193C allele had 1.45-fold lower P-glycoprotein levels in gut biopsies compared to those with homozygous 11156A and 11193T alleles. Moreover, Bosch *et al.*[14] recently identified 8 new allelic variants in the PXR gene and determined the allele frequencies of 24 PXR polymorphisms in 100 Dutch volunteers. Additionally, several studies already described an interaction between corticosteroids and tacrolimus however the exact mechanism

behind this interaction has never been elucidated[15-17]. In the present study, 26 early using corticosteroids and 37 late using no corticosteroids posttransplant patients are genotyped for PXR polymorphisms A7635G, C8055T, A11156C and T11193C. The genotypes of these renal transplant patients are associated with their corresponding pharmacokinetic parameters (dose-normalised) trough (C_0) concentration, (dose-normalised) area under the time concentration curve ($dnAUC_{0-12}$) and (dose-normalised) maximum concentration (dnC_{max}) to examine whether these PXR polymorphisms eventually in combination with the ude of corticosteroid have an influence on the inter-individual variability in the tacrolimus pharmacokinetics.

Patients and methods

Study population

In total 63 Caucasian renal transplant recipients divided over two different groups of whom in the past for clinical reasons (group I) or for a clinical trial (group II) a 12 hour time tacrolimus concentration curve was performed, were included in this study. Group I included early posttransplant patients that used corticosteroids with a short median time after transplantation and a large variability in the tacrolimus AUC_{0-12} compared to the late posttransplant recipients of group II that used no corticosteroids. Table 12.1 illustrates that most pharmacokinetic profiles of the patients included in group I were recorded within 6 weeks after transplantation while group II included patients that underwent a renal transplantation at least 1 year ago. Patients were not allowed to take food until 1 hour after ingesting the tacrolimus dose and were not allowed to take grapefruit juice after transplantation to prevent alterations in the tacrolimus metabolism. In addition, patients that suffer from gastrointestinal, liver disease or other disorders that may alter the absorption of tacrolimus were also disqualified for inclusion in both groups. Prior to the blood sample collection, there had been no tacrolimus dose change for at least three days in both groups. After overnight fasting the blood samples were collected immediately pre (C_0) and 0.5 ($C_{0.5}$), 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), 5 (C_5), 7.5 ($C_{7.5}$) and 12 (C_{12}) hours after the morning tacrolimus administration. Demographic as well as clinical data were determined at the time of recording the 12 hour time tacrolimus concentration curve. The renal transplant recipients of both groups underwent a renal transplantation in the University Hospital of Maastricht, the Netherlands.

Table 12.1 Demographic characteristics of the both renal transplant recipients groups.

Demographic characteristics	Group I	Group II
Gender (male/female)	18/8	24/13
Age (years, mean ± SD)	43.0 ± 13.2	51.3 ± 10.9
Length (cm, mean ± SD)	172 ± 8.2	174 ± 8.4
Weight (kg, mean ± SD)	69.1 ± 15.9	77.4 ± 13.5
Body Mass Index (kg/m^2, mean ± SD)	23.3 ± 4.40	25.6 ± 3.42
Primary kidney disease		
Glomerulonephritis	4	1
Chronic pyelonephritis	2	2
IgA nephropathy	3	4
Hypertensive nephropathy	4	7
Diabetes Mellitus nephropathy	4	0
Polycystic kidney disease	1	8
Unknown	1	4
Other	7	11
Transplantation number		
First	20	30
Second	5	6
Third or more	1	1
Tacrolimus mono therapy	1	29
Tacrolimus dose (mg/kg/day, mean ± SD)	0.39 ± 0.231	0.054 ± 0.029
C_0 (ng/ml, mean ± SD)	16.8 ± 5.83	6.59 ± 1.39
$AUC_{0\text{-}12}$ (ng × hr/ml, mean ± SD)	305.0 ± 96.8	122.5 ± 31.1
C_{max} (ng/ml, mean ± SD)	56.3 ± 21.3	20.9 ± 6.5
T_{max} (hr, mean ± SD)	1.46 ± 1.33	1.24 ± 0.43
Use of azothiopurine, MMF[a], rapamycine, steroids	11/3/4/26	3/4/0/0
Current steroid dose (mg, dose, no. patients)		
0 mg/day	3	37
5 mg/day	2	0
8 mg/day	2	0
10 mg/day	10	0
15 mg/day	4	0
20 mg/day	3	0
> 20 mg/day	2	0
Time since transplantation (days, median, (range))	18 (3-74)	1465 (453–4128)
Haemoglobin (mmol/l, mean ± SD)	5.43 ± 1.08	8.52 ± 0.83
Haematocrit fraction (mean ± SD)	0.25 ± 0.07	0.41 ± 0.04
ALAT (U/l, mean ± SD)	34 ± 34	24 ± 13
ASAT (U/l, mean ± SD)	22 ± 18	17 ± 10
Serum albumin (g/l, mean ± SD,)	30.7 ± 3.86	37.0 ± 3.84
Serum creatinine (μmol/l, median ± SD)	331 ± 293	125 ± 29
Creatinine (Cockcroft–Gault; ml/min, median ± SD)	37.5 ± 28.4	56.0 ± 26.6

[a] mycophenolate mofetil

Ethics

The study was performed in accordance to the Declaration of Helsinki and its amendements. The protocol was approved by the local Medical Ethics Committee and written informed consent for participation in this study was obtained from all patients.

Tacrolimus concentration determinations

The tacrolimus blood concentrations were determined in ethylene diamine tetra-acetic acid (EDTA) whole blood, using a microparticle enzyme immunoassay with a monoclonal antibody (IMx II assay; Abbott Laboratories, Abbott Park, IL, USA) for group I and a method based on LC tandem mass spectrometry (MS/MS) for group II. Both methods were performed in laboratories that participate in the International Tacrolimus Proficiency Testing Scheme. The tacrolimus C_0 concentration, the peak blood concentration (C_{max}) during the assessed time interval and the time at which the highest blood concentration was observed (T_{max}) were determined directly from the time *versus* tacrolimus blood concentration data. Additionally, the area under the time concentration curve (AUC_{0-12}) was calculated from the time *versus* tacrolimus concentration plot using the linear trapezoidal rule in MWPharm 3.50 (Mediware, Groningen, the Netherlands). DnC_0, $dnAUC_{0-12}$ and dnC_{max} were calculated by dividing the C_0, AUC_{0-12} and C_{max} respectively by the corresponding morning dose on a milligram per kilogram basis.

DNA isolation

Genomic DNA isolation was performed by using 200 μl EDTA anticoagulated blood for isolation with a QIAamp blood mini kit (Qiagen, Leusden, the Netherlands) according to the manufacturers' instructions.

Genotyping of PXR polymorphisms

Real-time PCR fluorescence resonance energy transfer (FRET) assays on the LightCycler (Roche Diagnostics, Almere, the Netherlands) were used for genotyping, PXR polymorphisms A7635G, C8055T, A11156C and T11193C using the same primers and probes compared to a previous publication (Op den Buijsch *et al.*, submitted). For each polymorphism, heterozygote samples were sequenced according to a direct sequence procedure on a capillary sequencer ABI Prism 3100 using the Bridge version 1.1 sequence kit (both products from Applied Biosystems, Fostercity, USA) and used in every real-time PCR FRET assay run as control sample.

Statistical analysis

Statistical analysis of the data was performed with use of the statistical software SPSS 11.0 for windows (Chicago, IL, USA). To examine the population homogeneity of the patients, the genotype frequencies of the PXR polymorphisms were tested against Hardy-Weinberg equilibrium by the Pearson's goodness-of-fit test[18]. For analysis of the daily tacrolimus dose (mg/kg/day), dnC_0 (ng/ml per mg/kg), $dnAUC_{0-12}$ (ng × hr/ml per mg/kg), dnC_{max} (ng/ml per mg/kg) and T_{max} (hr), groups were compared using non parametric statistical tests. To compare two groups we used the Mann-Whitney test, and to compare several groups the Kruskal Wallis test. *P* values less than 0.05 were

considered statistically significant. All values are expressed as median and range unless stated otherwise.

Results

The distribution of allele frequencies of four PXR polymorphisms

The clinical characteristics of the early posttransplant group using corticosteroids (group I) and a late posttransplant group using no corticosteroids (group II) that were enrolled in our study are shown in Table 12.1. Table 12.2 and 12.3 show the genotype frequencies of the different PXR polymorphisms that were determined. After genotyping the renal transplant patients for four PXR polymorphisms, the frequency of the 7635AA genotype is 36.5% (n = 23), for the 7635AC genotype 50.8% (n = 32) and for the 7635GG 12.7% (n = 8). In addition, the frequency of the 8055CC genotype is 68.3% (n = 43), for the 8055CT genotype 30.2% (n = 19) and for the 8055TT genotype 1.5% (n = 1) whereas the genotypes for the PXR polymorphisms A11156C and T11193C are in complete concordance with the genotypes of the PXR C8055T polymorphism. There is a complete haplotype between the following three PXR polymorphisms C8055T, A11156C and T11193C. The allele frequencies of the Caucasian renal transplant patients examined were found to be 31.7% (7635G) and 16.7% (8055T, 11156C and 11193T) which is in line with those published in previous studies[13,14]. The genotype frequencies of both renal transplant recipient groups were not significantly different from that predicted by the Hardy-Weinberg equation.

Table 12.2 Influence of PXR allelic variants on the pharmacokinetic tacrolimus parameters of the early posttransplant recipients in group I.

Genotype	N No	Allelic status	Dose (mg/kg/day)	DnC_0 (ng/ml per mg/kg)	$DnAUC_{0\text{-}12}$ (ng × hr/ml per mg/kg)	DnC_{max} (ng/ml per mg/kg)
PXR	6	A/A	0.464 (0.25-0.79)	83.7 (40-123)	1526 (824-2066)	236 (187-454)
A7635G	16	A/G	0.259 (0.04-0.80)	90.9 (42-1329)	1801 (839-15721)	400 (89-1535)
	4	G/G	0.420 (0.34-0.78)	60.8 (32-76)	1298 (922-1423)	332 (184-380)
PXR	16	C/C	0.271 (0.04-0.79)	110.5 (40-1329)[b]	1858 (824-15721)[a]	402 (176-1535)[b]
C8055T	10	C/T	0.435 (0.07-0.80)	63.7 (32-421)[b]	1244 (839-5954)[a]	293 (89-686)[b]
	0	T/T	-----	-----	-----	-----
PXR	16	A/A	0.271 (0.04-0.79)	110.5 (40-1329)[b]	1858 (824-15721)[a]	402 (176-1535)[b]
A11156C	10	A/C	0.435 (0.07-0.80)	63.7 (32-421)[b]	1244 (839-5954)[a]	293 (89-686)[b]
	0	C/C	-----	-----	-----	-----
PXR	16	T/T	0.271 (0.04-0.79)	110.5 (40-1329)[b]	1858 (824-15721)[a]	402 (176-1535)[b]
T11193C	10	T/C	0.435 (0.07-0.80)	63.7 (32-421)[b]	1244 (839-5954)[a]	293 (89-686)[b]
	0	C/C	-----	-----	-----	-----

Values are indicated as median and (range), [a] $P < 0.05$; [b] $P < 0.10$ (Mann-Whitney).

Table 12.3 Influence of PXR allelic variants on the pharmacokinetic tacrolimus parameters of the late posttransplant recipients in group II.

Genotype	N	Allelic	Dose	DnC0	DnAUC0-12	DnCmax
	No	status	(mg/kg/day)	(ng/ml per mg/kg)	(ng × hr/ml per mg/kg)	(ng/ml per mg/kg)
PXR	17	A/A	0.051 (0.02-0.13)	254 (70-669)	4973 (1355-11994)	820 (294-1729)
A7635G	16	A/G	0.049 (0.02-0.14)	286 (87-640)	4642 (1662-11504)	642 (308-1493)
	4	G/G	0.042 (0.03-0.07)	257 (121-598)	4488 (2766-9320)	814 (504-1086)
PXR	27	C/C	0.044 (0.02-0.13)	275 (70-669)	4761 (1355-11994)	795 (294-1729)
C8055T	9	C/T	0.052 (0.02-0.14)	278 (87-416)	4510 (1662-6854)	646 (308-981)
	1	T/T	0.038	223	4465	810
PXR	27	A/A	0.044 (0.02-0.13)	275 (70-669)	4761 (1355-11994)	795 (294-1729)
A11156C	9	A/C	0.052 (0.02-0.14)	278 (87-416)	4510 (1662-6854)	646 (308-981)
	1	C/C	0.038	223	4465	810
PXR	27	T/T	0.044 (0.02-0.13)	275 (70-669)	4761 (1355-11994)	795 (294-1729)
T11193C	9	T/C	0.052 (0.02-0.14)	278 (87-416)	4510 (1662-6854)	646 (308-981)
	1	C/C	0.038	223	4465	810

Values are indicated as median and (range).

Influence of the different PXR allelic variants on the pharmacokinetic tacrolimus profiles

The early posttransplant recipients carrying a PXR 8055T, 11156C and 11193T variant allele have a significantly lower and clinical relevant $dnAUC_{0\text{-}12}$ (1858 *versus* 1243 ng × hr/ml per mg/kg; Mann-Whitney, $P = 0.040$) or a strong trendency towards a lower dnC_0 (111 *versus* 64 ng/ml per mg/kg; Mann-Whitney, $P = 0.073$) or lower dnC_{max} (402 *versus* 293 ng/ml per mg/kg; Mann-Whitney, $P = 0.073$) compared to the carriers of the PXR 8055C, 11156A and 11193T wild type allele while in contrast no significant difference is observed regarding the pharmacokinetic parameters between carriers of the different PXR genotypes included in the corticosteroid group (group II). Figure 12.1A-D illustrate the effect of the different PXR polymorphisms on the $dnAUC_{0\text{-}12}$ recorded in 26 early and 37 late posttransplant recipients. Furthermore, the PXR haplotypes consisting of four polymorphisms are associated with the $dnAUC_{0\text{-}12}$ for the early as well as the late posttransplant patients, as is shown in Figure 12.1E and 12.1F. However, previously Op den Buijsch *et al.* (Op den Buijsch *et al.*, submitted) demonstrated in the same renal transplant patient group an important impact of the CYP3A5 A6986G polymorphism on the tacrolimus concentrations whereas no association was reported between both ABCB1 polymorphisms G2677T/A and C3435T and the tacrolimus concentration. To exclude influence of one of these polymorphisms, the renal transplant recipients were based on their CYP3A4 A-392G and CYP3A5 A6986G genotype categorized in order to examine solely the effect of the four PXR polymorphisms on the $dnAUC_{0\text{-}12}$. As is illustrated in Figure 12.2, there is a trend visible of the PXR C8055T, A11156C and T11193C after categorizing the patients for

respectively the CYP3A4 A-392G and CYP3A5 A6986G polymorphisms. Additionally, Table 12.4 and 12.5 illustrate the influence of the combination of respectively CYP3A4 A-392G and CYP3A5 A6986G with the two PXR polymorphisms A7635G and C8055T.

Table 12.4 Influence of the combination of PXR and CYP3A4, CYP3A5 allelic variants on the pharmacokinetic tacrolimus parameters of the early posttransplant recipients in group I.

Genotype combinations	N No	Allelic status	Dose (mg/kg/day)	DnC_0 (ng/ml per mg/kg)	$DnAUC_{0-12}$ (ng × hr/ml per mg/kg)	DnC_{max} (ng/ml per mg/kg)
PXR/CYP3A4	3	AA-AA	0.28 (0.25-0.72)	114 (40-123)	1920 (824-2066)	386 (187-454)
A7635G/A-392G	16	AG-AA	0.26 (0.04-0.80)	91 (42-1329)	1801 (839-15721)	400 (89-1535)
	2	GG-AA	0.35 (0.34-0.35)	69 (63-76)	1372 (1321-1423)	353 (327-380)
	3	AA-AG	0.50 (0.43-0.79)	60 (55-107)	1333 (1220-1796)	216 (212-256)
	2	GG-AG	0.63 (0.49-0.78)	46 (32-59)	1098 (922-1275)	261 (184-338)
PXR/CYP3A5	1	AA-GG	0.78*	32*	922*	184*
A7635G/A6986G	4	AA-AG	0.61 (0.43-0.79)*	58 (40-107)*	1277 (824-1796)*	214 (187-256)*
	3	AG-AG	0.64 (0.45-0.80)*	50 (42-65)*	1042 (864-1167)*	172 (89-260)*
	2	GG-AG	0.42 (0.35-0.49)*	61 (59-63)*	1298 (1275 -1321)*	359 (338-380)*
	3	AA-GG	0.28 (0.25-0.54)*	114 (45-123)*	1920 (839-2066)*	386 (90-454)*
	12	AG-GG	0.23 (0.04-0.59)*	174 (51-1329)*	2455 (1103-15721)*	473 (176-1535)*
	1	GG-GG	0.34*	76*	1423*	327*
PXR /CYP3A4	13	CC-AA	0.25 (0.04-0.72)**	123 (40-1329)	2030 (824-15721)	454 (176-1535)**
C8055T/A-392G	9	CT-AA	0.42 (0.07-0.80)**	65 (42-421)	1321 (839-5954)	327 (89-686)**
	3	CC-AG	0.50 (0.43-0.79)**	60 (55-107)	1333 (1220-1796)	216 (212-256)**
	1	CT-AG	0.78**	32	922	184**
PXR/CYP3A5	1	CT-AA	0.78*	32*	922*	184*
C8055T/A6986G	5	CC-AG	0.50 (0.43-0.79)*	59 (40-107)*	1275 (824-1796)*	216 (187-338)*
	4	CT-AG	0.55 0.35-0.80)*	53 (42-65)*	1132 (864-1321)*	213 (89-380)*
	11	CC-GG	0.25 (0.04-0.59)*	167 (51-1329)*	2066 (1103-15721)*	471 (176-1535)*
	5	CT-GG	0.34 (0.07-0.54)*	90 (45-421)*	1715 (839-5954)*	381 (90-686)*

Values are indicated as median and (range), * $P < 0.05$; ** $P < 0.10$ (Kruskal Wallis).

Table 12.5 Influence of the combination of PXR and CYP3A4, CYP3A5 allelic variants on the pharmacokinetic tacrolimus parameters of the late posttransplant recipients in group II.

Genotype combinations	N No	Allelic status	Dose (mg/kg/day)	DnC_0 (ng/ml per mg/kg)	$DnAUC_{0-12}$ (ng×hr/ml per mg/kg)	DnC_{max} (ng/ml per mg/kg)
CYP3A4/PXR	17	AA-AA	0.05 (0.02-0.13)	254 (70-669)	4973 (1355-11994)	820 (294-1729)
A7635G/A-392G	16	AG-AA	0.05 (0.02-0.14)	286 (87-640)	4642 (1662-11504)	642 (308-1493)
	3	GG-AA	0.04 (0.03-0.05)	289 (223-598)	4511 (4465-9320)	818 (810-1086)
	1	GG-AG	0.07	121	2766	504
PXR/CYP3A5	1	AA-AG	0.13	70**	1355**	294**
A7635G/A6986G	3	AG-AG	0.10 (0.05-0.14)	87 (87-248)**	1663 (1662-4057)**	461 (308-646)**
	1	GG-AG	0.07	121**	2766**	504**
	16	AA-GG	0.05 (0.02-0.11)	264 (0.02-0.11)**	4991 (2227-11994)**	842 (495-1729)**
	13	AG-GG	0.04 (0.02-0.07)	295 (128-640)**	4761 (2673-11504)**	643 (465-1493)**
	3	GG-GG	0.04 (0.03-0.05)	290 (223-598)**	4511 (4465-9320)**	818 (810-1086)**
PXR /CYP3A4	26	CC-AA	0.04 (0.02-0.13)	279 (70-669)	4867 (1355-11994)	808 (294-1729)
C8055T/A-392G	9	CT-AA	0.05 (0.02-0.14)	278 (87-416)	4511 (1662-6854)	646 (308-981)
	1	TT-AA	0.04	223	4465	810
	1	CC-AG	0.07	121	2766	504
PXR/CYP3A5	3	CC-AG	0.10 (0.07-0.13)**	87 (70-121)*	1663 (1355-2766)*	461 (294-504)*
C8055T/A6986G	2	CT-AG	0.10 (0.05-0.14)**	168 (87-248)*	2859 (1662-4057)*	477 (308-646)*
	24	CC-GG	0.04 (0.02-0.11)**	289 (118-669)*	4992 (2227-11194)*	842 (495-1729)*
	7	CT-GG	0.05 (0.02-0.07)**	290 (128-416)*	4567 (2673-6854)*	676 (465-981)*
	1	TT-GG	0.04**	223*	4465*	810*

Values are indicated as median and (range), *P < 0.05; **P < 0.10 (Kruskal Wallis).

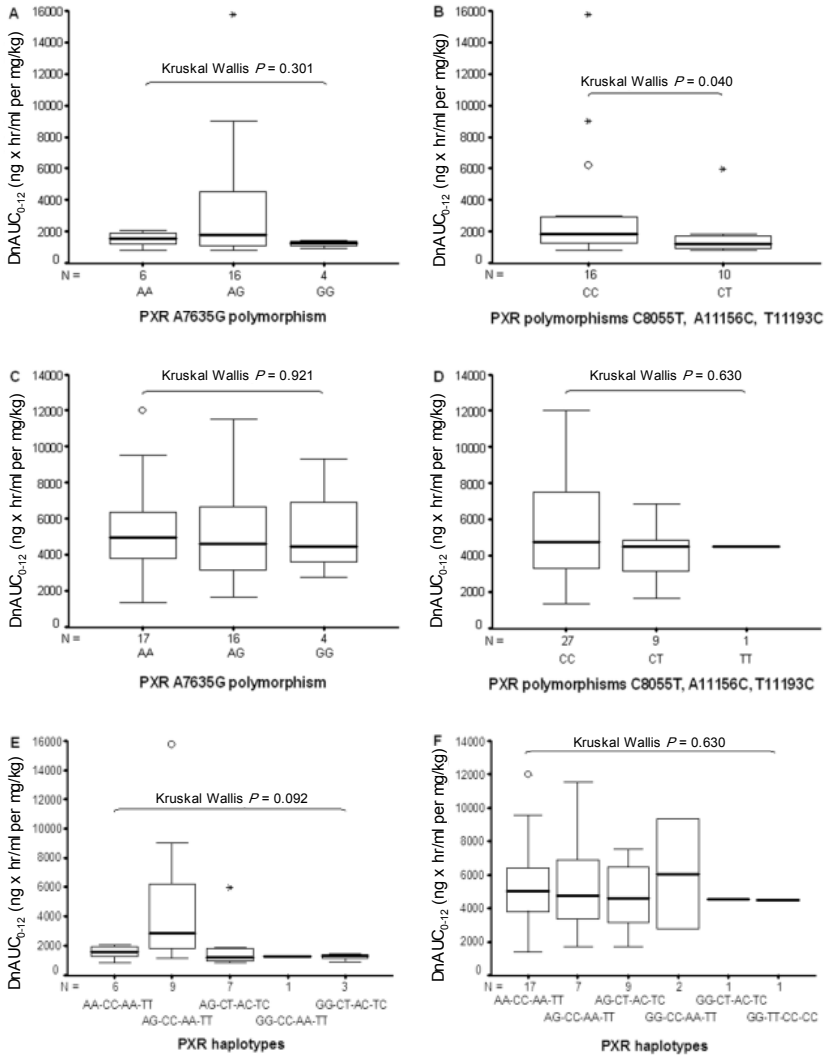

Figure 12.1 Influence of the PXR A7635G, C8055T, A11156C and T11193C genotypes on the area under the time tacrolimus concentration curve (AUC_{0-12}) that were recorded in 26 early and 37 late posttransplant patients. Figure 12.1 illustrates the boxplots of the distribution of the dose-normalized AUC_{0-12} (ng × hr/ml per mg/kg) clustered according to the PXR A7635G genotype, PXR C8055T, A11156C and T11193C genotype for the early posttransplant patient group (A and B) and the late posttransplant patient group (C and D). Figure 12.1 E and F show the boxplots of the distribution of the dose-normalized AUC_{0-12} (ng × hr/ml per mg/kg) clustered according to the PXR haplotypes consisting of the PXR polymorphisms A7635G, C8055T, A11156C and T11193C in respectively the early and the late posttransplant patient group. Open circles indicate an outlier value at more than 1.5 box lengths above the box while asterices indicate an extreme value at more than 3 box lengths above the box.

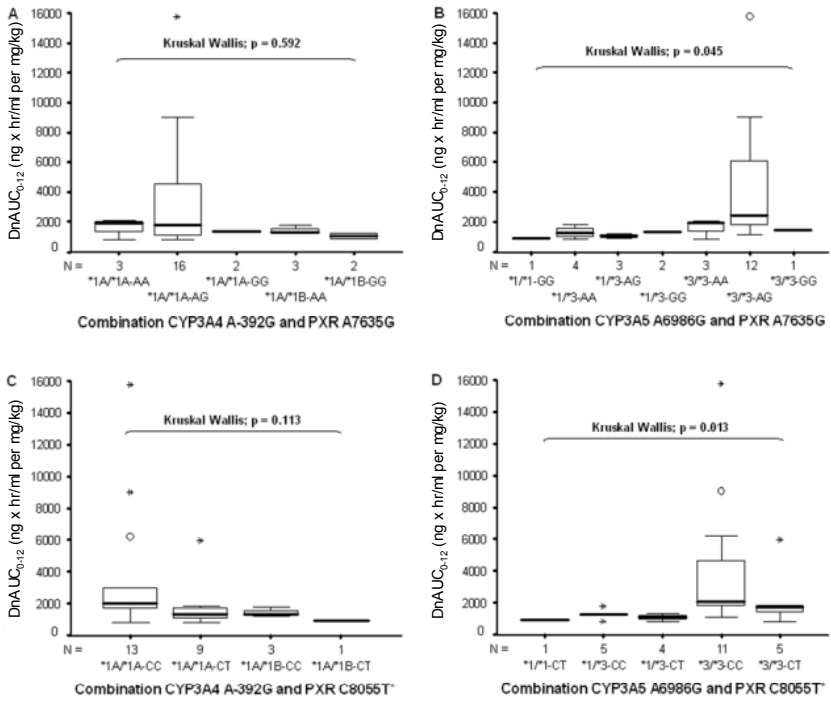

Figure 12.2 Figure 12.2 illustrates the boxplots of the distribution of the dose-normalised $AUC_{0\text{-}12}$ (ng × hr/ml per mg/kg) clustered according to respectively (A) the combination of CYP3A4 A-392G and PXR A7635G, (B) the combination of CYP3A5 A6986G and PXR A7635G, (C) the combination of CYP3A4 A-392G and PXR C8055T and (D) the combination of CYP3A5 A6986G and PXR C8055T in the early posttransplant patient group. * indicates that the PXR polymorphisms A11156C and T11193C show exactly the same figures. Open circles indicate an outlier value at more than 1.5 box lengths above the box while asterices indicate an extreme value at more than 3 box lengths above the box.

Discussion

After genotyping 63 renal transplant patients divided into 26 early posttransplant recipients and 37 late posttransplant recipients it appeared that there is a complete PXR haplotype consisting of the polymorphisms C8055T, A11156C and T11193C. Although the genotypes of the four PXR polymorphisms differed not significantly between the early posttransplant recipient group and the late posttransplant recipient group, there is a difference in response between the different allelic variants of the PXR polymorphisms. The carriers of variant PXR alleles in the early renal transplant recipients showed a significant trend towards lower pharmacokinetic parameters (dnC_0,

dnAUC$_{0\text{-}12}$ and dnC$_{max}$) compared to carriers of wild type PXR alleles. However, patients included in the late posttransplant group that carry a variant PXR allele showed no trend towards lower pharmacokinetic parameters for tacrolimus compared to the patients that carried wild type alleles. Almost all renal transplant recipients included in group I used steroids as an additive immunosuppressivum while no of the 37 renal transplant recipients included in group II used corticosteroids. Previously, van Duijnhoven *et al.*[15] first described an increase of the tacrolimus C_0 concentration after corticosteroid withdrawal while two other studies[16,17] also described a similar interaction between corticosteroids and tacrolimus. Induction of PXR by corticosteroids may lead to a higher expression of PXR and thereby to a higher expression of CYP3A4 and ABCB1 which may result in a higher daily tacrolimus dosage to maintain the pharmacokinetic parameters of tacrolimus within the therapeutic range. Differences in the tacrolimus C_0 concentrations after administrating corticosteroids or withdrawing corticosteroids in transplant patients may be explained by polymorphisms present in the PXR gene. Zhang *et al.*[13] demonstrated for the variant allele of the two PXR polymorphisms A7635G and C8055T a trend towards a higher of intestinal CYP3A after induction with rifampicin. In addition, a tendency was reported to lower P-glycoprotein concentrations regarding carriers of the 3'-untranslated region variant alleles of the PXR polymorphisms 11156A to C and 11193T to C. Our findings indicate that there is an inclination towards a lower dnC$_0$, dnAUC$_{0\text{-}12}$ and dnC$_{max}$ for patients who are carrier of at least one variant allele of the PXR polymorphisms examined. Remarkably, this trend was only observed in the early posttransplant patient group while the genotypes of the different PXR polymorphisms showed no trends towards lower pharmacokinetic parameters in the late posttransplant patient group. Due to the lack of corticosteroid use in the late posttransplant recipient group, the influence of PXR polymorphisms on the pharmacokinetic parameters of tacrolimus appears to be minor which may explain the discrepancy between the early and the late posttransplant recipient group. Summarised, tacrolimus is predominantly metabolised by the CYP3A iso-enzymes while the ABCB1 modulates its bioavailability. Although there is a high inter-individual variation in the expression of CYP3A4 and ABCB1, genetic polymorphisms can not exclusively explain the difference in CYP3A4 and ABCB1 expression. PXR has been identified as a transcriptional regulator of CYP3A4 and ABCB1. In the present study, four variant alleles of PXR polymorphisms A7635G, C8055T, A11156C and T11193C show a trend towards lower pharmacokinetic parameters however only in the early posttransplant patient group and not in the late posttransplant patient group. Since PXR polymorphisms demonstrate an effect in the early posttransplant group we hypothesised that the PXR polymorphisms have only an effect on the pharmacokinetic parameters of tacrolimus after induction by e.g. corticosteroids. Our findings indicate that PXR polymorphisms may play a role in tacrolimus metabolism although their role is dependent of the presence and/or of inductive drug or chemical components in the transplant patients.

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