Results

After measuring the QC samples for a longer period, we concluded that most data points were within the process mean ± 2 standard deviations (SD) and none of the points were outside the process mean ± 3 SD range. On the basis of those results, we defined the following acceptance criteria, data points should be in the established range of the process mean ± 2 SD for the *m/z* values, peak intensities, S/N ratios, and peak resolutions for insulin, apomyoglobin and albumin in the QC samples. Figure 1 shows a graphic and a two and three dimensional Youden plot generated with the software.

In this study was demonstrated that variations in the signal of the QC samples can be caused by pipetting variability in the handling of the QC sample, spot and chip variability, crystallization of the energy absorbing matrix, and laser detector variability over time.

Conclusions

Any new technology, particularly one being presented as a potential clinically used diagnostic tool, requires stringent quality control to evaluate analytical performance over time. Instrument performance, however, must be compared not only during one experiment, but also over the course of time. In this study a standard protocol for calibration was defined and acceptance criteria for the independent certified QC samples were established. The composition of the QC samples was based on the way of calibration. Insulin, apomyoglobin, and albumin were chosen to validate the peptide and protein-low calibration equations, respectively. By checking the calibration every week, the QC procedure acts prospectively, while in some studies the quality control acts retrospectively by including the QC samples in the profiling experiments and in some studies there is no quality control procedure described at all. Stringent QC prevents unreliable data acquisition from the very start and when the data of the QC samples exceed the acceptance criteria, actions needs to be undertaken before starting new protein profiling experiments.

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Influence of cytochrome P450 3A5 and multidrug resistance-1 gene single nucleotide polymorphisms (SNPs) on the tacrolimus area under the curve (AUC) in renal transplant recipients

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Tacrolimus is used worldwide for primary immunosuppression following renal transplantation. Moreover, tacrolimus has a narrow therapeutic index, which makes close therapeutic drug monitoring necessary to prevent both sub-therapeutic blood levels and toxic blood levels. Sub-therapeutic tacrolimus blood levels increase the risk of transplant rejection (1, 2), while toxic tacrolimus blood levels may lead to severe side effects such as nephrotoxicity and neurotoxicity (3). The high interindividual variability in tacrolimus pharmacokinetics complicates the realization of this narrow therapeutic index.

Therapeutic drug monitoring of tacrolimus can be performed in the most exact way by making 12-hour pharmacokinetic profiles. Recent studies (4, 5) showed

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higher correlations with the 12-hour pharmacokinetic profiles of tacrolimus when a limited sampling point sample strategy is used instead of trough (C_0) levels alone. In the present study, a two time point sample strategy is used to calculate the area-under-the-time-tacrolimus-concentration curve or $AUC_{0\text{-}12}$ of 103 Chinese renal transplant recipients.

It is well known that the cytochrome P450 (CYP) 3A iso-enzymes mainly represented by CYP3A4 and CYP3A5 have been identified as the major enzymes responsible for the metabolism of tacrolimus (6). The most described single nucleotide polymorphism (SNP) in CYP3A5 is 6986G or *3 which displays a sequence variability in intron 3 that creates a cryptic splice site and encodes an aberrantly spliced mRNA with a premature stop codon, leading to the absence of protein expression. This CYP3A5*3 polymorphism occurred homozygously in 90% of the Caucasians, 73% of the Chinese and in 30% of the African-American population (7). Tacrolimus is also a substrate for P-glycoprotein (P-gp) which is the product of the multidrug resistance-1 (MDR1) gene. Three partly linked polymorphisms in the MDR1 gene located on exons 12, 21 and 26 have been studied widely and these polymorphisms account for the major haplotypes encountered in Caucasians (8).

In the present study the impact is examined of CYP3A5 and MDR1 polymorphisms on the dose-normalized (dn)$AUC_{0\text{-}12}$ which is calculated according to a two time point sampling strategy.

Material and methods

A total of 103 Chinese renal transplant recipients who received tacrolimus as part of the immunosuppressive therapy and had regular follow up in Queen Elizabeth Hospital or Tuen Mun Hospital in Hong Kong were included in this retrospective study. There was no change in the daily tacrolimus dose for at least two weeks. Patients who were taking medication known to have interaction with tacrolimus, such as calcium channel blockers, anti-epileptics, anti-mycotics and macrolide antibiotics were excluded from this study. Additionally, patients who suffered from gastrointestinal disease, liver disease or other disorders that may alter the absorption of tacrolimus were also excluded. Apart from tacrolimus and steroid, these patients were normally put on azathioprine, however some patients preferred to use mycophenolic acid on advice of their private physician.

The dosage of azathioprine was 1.5 mg/kg/day while the dosage of mycophenolic acid was 0.5 gram twice daily. The initial tacrolimus dosage, administrated twice daily, was 0.3 mg/kg per day for all patients. The daily tacrolimus dose was then adjusted according to the $AUC_{0\text{-}12}$ value, which was kept at around 100-150 ng×hr/mL in the first 3 months. After three months the target $AUC_{0\text{-}12}$ value was decreased to around 80-100 ng×hr/mL for long-term maintenance. The steroid regimen for the first month was 30 mg/day of oral prednisolone, progressively tapered by 2.5 mg every two weeks until a daily maintenance dose of 5 mg.

Tacrolimus blood concentrations were determined 2 (C2) and 4 (C4) hours after the morning tacrolimus administration in ethylene diamine tetra acetic acid (EDTA) whole blood using a semi-automated microparticle enzyme immunoassay (MEIA) on an IMX II clinical analyser (Abbott Laboratories, Abbott Park, IL, USA). The two tacrolimus blood concentrations determined were used to calculate the $AUC_{0\text{-}12}$ according to the equation based strategy as described earlier (4): $AUC_{0\text{-}12} = 16.2 + 2.4 \times C2 + 5.9 \times C4$. The $dnAUC_{0\text{-}12}$ was calculated by dividing the $AUC_{0\text{-}12}$ by the corresponding 24 hour dose on a milligrams per kilogram basis. The study was performed in accordance to the Declaration of Helsinki and its amendments. The protocol was approved by the Medical Ethics Committee of the Queen Eliza-

Table 1. Influence of CYP3A5 and MDR1 genotypes on the daily tacrolimus dose and the dose-normalized (Dn)$AUC_{0\text{-}12}$

Genotype	Allelic status (n)	Dose (mg/kg/day)	$DnAUC_{0\text{-}12}$ (ng×hr/mL per mg/kg)
CYP3A5	*1/*1 (10)	0.090 (0.05-0.18)*	920 (300-1981)*
A6986G	*1/*3 (38)	0.070 (0.04-0.15)*	1228 (589-2266)*
	*3/*3 (55)	0.050 (0.01–0.10)*	2143 (827-11125)*
MDR1	C/C (8)	0.060 (0.03-0.15)	1484 (875-5166)
	C1236TC/T (52)	0.060 (0.01-0.18)	1494 (300-11125)
	T/T (43)	0.063 (0.01-0.15)	1651 (589-7579)
MDR1	G/G (26)	0.053 (0.03-0.09) **	1887 (891-6166)
	G2677T/AG/T (32)	0.070 (0.01-0.18)	1365 (300-7579)
	G/A (16)	0.050 (0.01-0.15)	1864 (676-11125)
	T/A (10)	0.056 (0.05-0.13)	1765 (805-2870)
	T/T (19)	0.066 (0.02-0.15) **	1473 (589-4532)
MDR1	C/C (46)	0.052 (0.01-0.15) **	1882 (676-11125)
C3435T	C/T (43)	0.063 (0.03-0.18)	1466 (300-6166)
	T/T (14)	0.073 (0.02-0.15)**	1332 (589-4532)

Values are indicated as median (range), *$P < 0.001$ (Kruskal-Wallis); **$P < 0.05$ (Mann-Whitney).

beth Hospital in Hong Kong and written informed consent for participation in this study was obtained from all patients.

Genomic DNA was extracted from 103 Chinese renal transplant recipients by using 200 µL EDTA anti-coagulated blood for isolation with a QIAamp blood mini kit (Qiagen, Leusden, the Netherlands) according to the manufacturers instructions. Real-time PCR fluorescence resonance energy transfer (FRET) assays were used for genotyping the MDR1 G2677T/A and C3435T polymorphisms with the same primers and probes as described in the original publications (9, 10). However, real-time PCR FRET assays for the CYP3A5 A6986G and MDR1 C1236T polymorphisms were designed and validated on our laboratory.

Results

Table 1 shows a significant decrease in the $dnAUC_{0\text{-}12}$, which strongly depends on whether the patients were carrier of none, one or two CYP3A5*1 alleles (2143, 1228 and 920 ng×hr/mL per mg/kg; Kruskal-Wallis, $P<0.001$), respectively. This allele-dependent effect of the CYP3A5*3 polymorphism is also illustrated in figure 1. Consequently, the daily tacrolimus dose was 80% higher in homozygous carriers of a CYP3A5*1 allele compared to homozygous carriers of the CYP3A5*3 allele (0.09 mg/kg/day *versus* 0.05 mg/kg/day; Kruskal-Wallis, $P<0.001$). The MDR1 polymorphisms G2677T/A and C3435T showed only a weak significant difference regarding the daily tacrolimus dose, while no significant difference is observed between these MDR1 polymorphisms and the $dnAUC_{0\text{-}12}$. No significant results were observed between MDR1 C1236T polymorphism and both the daily tacrolimus dose and pharmacokinetic tacrolimus parameters.

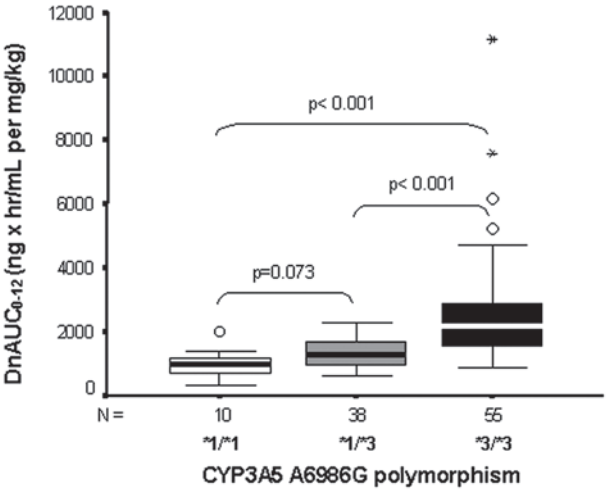

Figure 1. Influence of the CYP3A5 A6986G genotype on the pharmacological parameters recorded in 103 renal transplant recipients. The boxplot of the dose-normalized (dn)$AUC_{0\text{-}12}$ (ng×hr/mL per mg/kg body weight) clustered according to CYP3A5 A6986G genotype. ◇ Outlier value at more than 1.5 boxlengths above the box. * Extreme value at more than 3 box lengths above the box.

Conclusion

Knowing the impact of the CYP3A5*3 allele on the tacrolimus metabolism, it is certainly worthwhile considering to screen renal transplant recipients for this polymorphism in order to obtain a better optimised immunosuppressive therapy.

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