

## Effect of simvastatin on cyclosporine unbound fraction and apparent blood clearance in heart transplant recipients

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**Aims** To investigate the effects of lipid lowering therapy on the fraction unbound and dosage requirement of cyclosporine in heart transplant recipients.

**Methods** Cyclosporine fraction unbound (*f<sub>u</sub>*) was measured *ex vivo* in plasma obtained from heart transplant recipients (*n* = 12) before and after lipid lowering treatment, using equilibrium dialysis. Cyclosporine trough concentration data were also collected from cardiac transplant recipients (*n* = 32) who received simvastatin for the treatment of hyperlipidaemia. Cyclosporine daily dosage and total concentration (monoclonal FPIA method) were recorded for periods up to 6 months before and after simvastatin administration. The total number of dose rate-concentration observations was 172 before and 135 after simvastatin administration respectively. Using a population pharmacokinetic approach (implemented in P-PHARM software) the ratio of dose rate to trough concentration at steady state ( $DR/C_{ss\text{trough}}$ ), an estimation of apparent clearance, was determined. The posterior Bayesian estimate of  $DR/C_{ss\text{trough}}$  was calculated for each patient before and after simvastatin administration.

**Results** The mean *f<sub>u</sub>* increased by 29%, from  $1.40 \pm 0.1\%$  (mean  $\pm$  s.d.) to  $1.82 \pm 0.22\%$  after simvastatin administration ( $P < 0.01$ ). Mean trough concentrations of cyclosporine in whole blood were  $349 \mu\text{g l}^{-1}$  before and  $242 \mu\text{g l}^{-1}$  after simvastatin administration ( $P < 0.0001$ ). The mean cyclosporine daily dosage was  $2.87 \text{ mg kg}^{-1}$  and  $2.33 \text{ mg kg}^{-1}$  (NS), before and after simvastatin administration respectively. The average cyclosporine  $DR/C_{ss\text{trough}}$  was significantly increased from  $24.5 \text{ l h}^{-1}$  before to  $28.9 \text{ l h}^{-1}$  after simvastatin administration ( $P < 0.05$ ). Furthermore the median increase in cyclosporine  $DR/C_{ss\text{trough}}$  was  $18 \text{ l h}^{-1}$  ( $-3.1$  to  $42.1 \text{ l h}^{-1}$ , interquartile range).

**Conclusions** Cyclosporine fraction unbound and clearance are increased following co-administration of lipid lowering agents, necessitating closer monitoring of cyclosporine total blood concentration when lipid lowering agents are administered concomitantly with cyclosporine.

**Keywords:** cyclosporine, simvastatin, fraction unbound, heart transplant, population pharmacokinetics, clearance, lipoproteins, drug interaction, LDL cholesterol

### Introduction

Cyclosporine is an immunosuppressive agent widely used following organ transplantation [1]. It exhibits a high degree of binding to blood cells and plasma proteins having a plasma unbound fraction of approximately 2% [2, 3]. In plasma it is highly bound to lipoproteins including HDL and LDL and cyclosporine unbound fraction exhibits an inverse correlation with the concentration of serum cholesterol and triglyceride [4]. Transplant recipients are prone to hyperlipidaemia [5], which predisposes to transplant coronary artery disease which in turn is a major cause of death after cardiac transplantation [6, 7]. Lipid lowering therapy is commonly used in this patient group to treat hypercholesterolaemia in which HMG-CoA reductase inhibitors such as simvastatin [8, 9] or pravastatin [10] are the most commonly

used. These medications are likely to alter the concentration of serum total and LDL cholesterol which in turn may influence the fraction unbound of cyclosporine in plasma.

Cyclosporine has low to intermediate hepatic extraction [11] thus theoretically the clearance of cyclosporine may depend on the fraction of drug that is unbound in blood or plasma [12]. An increase in plasma lipoproteins may lead to a subsequent decrease in cyclosporine clearance and therefore an increase in cyclosporine concentrations [13, 14]. Furthermore deLogreril *et al.* [15] have shown that the administration of the lipid lowering agent, fenofibrate, to heart transplant recipients resulted in a slight increase in cyclosporine clearance when calculated from h.p.l.c. data. In these studies [13, 14, 15] cyclosporine unbound fraction was not measured. It was proposed however that hyperlipidaemia may decrease cyclosporine clearance by reducing the cyclosporine fraction unbound in plasma, and conversely the administration of lipid lowering agents may alter the fraction unbound resulting in increased cyclosporine clearance.

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The aim of this study was to investigate the influence of administration of simvastatin on cyclosporine fraction unbound, total concentration and apparent clearance in cardiac transplant recipients.

## Methods

### Patients

Samples of blood and cyclosporine dosage-concentration data were obtained as a part of a randomized clinical trial to administer ketoconazole as a cyclosporine metabolic inhibitor. The Research and Ethics Committee of St Vincent's Hospital approved the study and written consent was obtained from each patient participating in the trial.

**Study I** Cyclosporine unbound fraction was measured in plasma samples from 12 (four female and eight male) heart transplant recipients who received simvastatin. The mean weight was  $67 \pm 10.9$  kg (mean  $\pm$  s.d.) and the mean age was 52 years (47 to 60 years). The daily cyclosporine dosage was  $3.6 \pm 1.9$  mg kg<sup>-1</sup> and total cyclosporine blood concentration was  $226 \pm 72$  µg l<sup>-1</sup>. Concentrations of serum total, LDL and HDL cholesterol and total triglyceride were measured by an accredited laboratory (Chemical Pathology, St Vincent's Hospital, Victoria St, Darlinghurst, Sydney, Australia) using the standard enzymatic method. The daily dosage of simvastatin was 5 mg for two patients, 10 mg for nine patients and 15 mg for one patient.

**Study II** The case notes of 100 additional heart transplant patients were retrospectively reviewed and 32 heart transplant recipients who received lipid lowering therapy were found. The cyclosporine total daily dosage and total concentration measured in whole blood (trough concentration) for these patients were recorded before and after simvastatin administration and the data were examined using a population pharmacokinetic approach. Cyclosporine daily dosage and total cyclosporine concentration data for a period of time before and after administration of simvastatin was obtained for the 32 cardiac transplant recipients. Nine patients were female. The mean weight was 75.1 kg (55 to 111 kg) and the mean age was 50 years (28 to 64 years). The average time to initiation of lipid lowering therapy after transplantation was  $223 \pm 174$  days (range 25 to 734 days). The lipid lowering regimen was 5 mg simvastatin per day for 11 patients, 10 mg day<sup>-1</sup> for 20 patients and 40 mg day<sup>-1</sup> for one patient. A number of patients ( $n=20$ ) were also receiving ketoconazole 100 or 200 mg day<sup>-1</sup> as a cyclosporine sparing agent as part of a randomized trial [17].

### Analysis of cyclosporine fraction unbound

Cyclosporine fraction unbound was analysed using equilibrium dialysis and h.p.l.c. purified [<sup>3</sup>H]-cyclosporine to eliminate small amounts of radiochemical impurities.

**Purification of [<sup>3</sup>H]-cyclosporine** The chromatographic system consisted of a pre-column packed with silica (Pre-Sat<sup>TM</sup>, Alltech, IL, USA), a guard column packed with Licrosorb C<sub>18</sub> with an average particle size of 10 µm (E. Merck,

Darmstadt, Germany) and an analytical column Techsil C<sub>18</sub> (0.46 ID  $\times$  25 cm long) packed with 10 µm average particle size (HPLC Technology Ltd, Macclesfield, UK), all maintained at 65°C. The mobile phase was composed of acetonitrile:water (82:18) at a flow rate of 1.5 ml min<sup>-1</sup>. Detector wavelength was set at 214 nm. Samples of [Mebmt-β-<sup>3</sup>H]-cyclosporine (Amersham, Buckinghamshire, UK), containing 11.9 µCi of [<sup>3</sup>H]-cyclosporine (1.19 µCi µg<sup>-1</sup>) were dissolved in acetonitrile:methanol (1:1; 20 µl) and injected into the h.p.l.c. system. Eluent was collected when the cyclosporine peak was indicated by the u.v. detector, dried under a stream of nitrogen and reconstituted in isopropanol to produce appropriate concentrations. The purified [<sup>3</sup>H]-cyclosporine in isopropanol was added to plasma such that the final concentration of isopropanol was less than 0.2% v/v and to provide a total cyclosporine concentration of 135 µg l<sup>-1</sup>. The radioisotopic activity was approximately 10 000 d min<sup>-1</sup> for 100 µl of plasma sample.

**Equilibrium dialysis** Dialysis cells having a total volume of 1.36 ml per half cell were constructed with medical grade stainless steel (ULCO ENGINEERING, Sydney, Australia) to replace the original PTFE cells. Spectrum equilibrium dialysis apparatus and cellulose dialysis membranes with a molecular weight cut-off of 12 000–14 000 (Spectra/Por-2) (Spectrum Medical Industries Inc., Los Angeles, CA, USA) were used. Samples of plasma (1 ml) were spiked with radiolabelled cyclosporine and dialysed against isotonic phosphate buffer at 37°C for 18 h until equilibrium was established. After equilibration 100 µl aliquots of plasma or buffer were sampled simultaneously using two glass syringes (100 µl; Hamilton Co., Reno, Nevada, U.S.A.). The syringes were rinsed once with 100 µl water and twice with methanol (100 µl) and the rinsings were pooled with the sample. The radioisotopic activity was counted using a liquid scintillation counter (Tri-Carb model 19 000CA, Packard Instrument Company, Downers Heights, IL, USA). The volume shift was determined by measuring the total plasma protein concentration before and after dialysis using the biuret method [18]. The intra-day coefficient of variation of estimates of fraction unbound was less than 15% ( $n=16$ ).

### Population pharmacokinetic analysis

The ratio of dose rate to steady-state trough concentration ( $DR/C_{ss\text{trough}}$ ) was estimated using whole blood cyclosporine trough concentration data obtained from routine cyclosporine therapeutic drug monitoring. Total cyclosporine levels in whole blood, were measured by the Department of Clinical Pharmacology, St Vincent's Hospital. The assay method was monoclonal TDx<sup>®</sup> method (Abbott Laboratories, Abbott Park, IL) utilising fluorescence polarisation immunoassay (FPIA) technology. Apparent clearance ( $CL/F$ ) of drugs at steady state can be estimated from Equation 1 [19]

$$DR = \frac{CL}{F} \times \overline{C_{ss}} \quad (1)$$

Where DR is dose rate,  $CL/F$  is apparent clearance,  $F$  is the bioavailability and  $\overline{C_{ss}}$  is the average concentration at steady state. This equation has been used by Grevel *et al.*

[20] to examine the population pharmacokinetic of cyclosporine in renal transplant recipients. In this study only blood concentrations at the end of the dosage interval were available, therefore it was assumed that the ratio of dose rate and steady-state trough concentration ( $DR/C_{ss\text{trough}}$ ) and not the average concentration at steady-state can be considered as a close approximation of apparent clearance (Equation 2).

$$DR = \theta \times C_{ss\text{trough}} \quad (2)$$

Where  $\theta$  ( $1\text{h}^{-1}$ ) is the ratio of  $DR/C_{ss\text{trough}}$ . Steady state was assumed to be achieved after 5 half-lives (approximately 2 days) of oral cyclosporine administration at the same dose rate. The average time interval between the last cyclosporine dosage and collection of the blood samples was  $12 \pm 1$  h (mean  $\pm$  s.d.). Dose rate-concentration data were analysed using a population pharmacokinetic software package (P-PHARM<sup>TM</sup>, ver 1.3, SIMED, France). Equation 2 was used to calculate cyclosporine dose rate/ $C_{ss\text{trough}}$ . In this study we choose to use a mixed effects modelling approach to determine the population mean and variability of the  $DR/C_{ss\text{trough}}$  parameter. Preliminary analysis of the distribution of residuals and the reduction in standard deviation of the parameter estimates indicated that the interindividual variability was best described by a log-normal rather than a normal distribution. Also a heteroscedastic error model (proportional to the squared value of the inverse prediction), rather than a homoscedastic error model best described the residual variability in the prediction of dose rate. P-PHARM generates posterior Bayesian parameter estimates for  $DR/C_{ss\text{trough}}$  for each subject which can be compared to patient specific factors including type of cyclosporine sparing agent, using stepwise multiple linear regression. This approach selects a covariable or combination of covariables and examines the relationship with pharmacokinetic parameters using a partial  $F$  test to judge the statistical significance. The population mean  $DR/C_{ss\text{trough}}$  was estimated for each data set and subsequently posterior Bayesian estimates of  $DR/C_{ss\text{trough}}$  were calculated for each subject to allow examination of the effect of covariates on  $DR/C_{ss\text{trough}}$ .

#### Statistical analysis

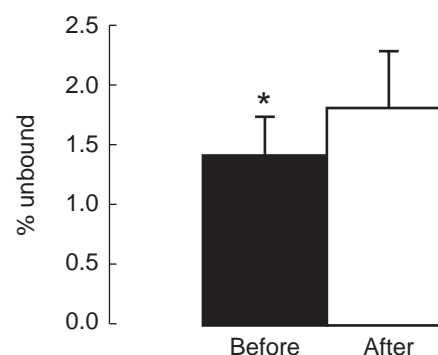
Statistical analyses were performed using SPSS<sup>®</sup> for Windows<sup>TM</sup> (Release 6.1, SPSS Inc.). Unless otherwise stated,

all values of  $P$  are based on two-tailed tests and  $P$  values less than 0.05 were considered significant. To establish if the data were normally distributed Kolmogorov-Smirnov test for goodness of fit was performed on every variable. Significance of a difference in means for two independent samples was examined using Student's  $t$  statistics (independent sample  $t$ -test) or Mann-Whitney  $U$ -test. For paired samples, paired-sample  $t$ -test and the Wilcoxon test were used.

#### Results

The composition of data sets for study I and II is displayed in Table 1. The mean percent unbound of cyclosporine in plasma increased by 29%, from  $1.40 \pm 0.1\%$  (s.d.) to  $1.82 \pm 0.22\%$  after simvastatin administration (Figure 1). Mean values of cyclosporine fraction unbound at various time intervals before and after the initiation of lipid lowering treatment are displayed in Figure 2. The data show an increase in the values of percentage unbound with respect to time after lipid lowering treatment is commenced.

The concentrations of serum lipoproteins in the 12 transplant patients (study I data) before and after lipid lowering therapy with simvastatin are shown in Figure 3. The concentration of total and LDL-cholesterol were reduced as a result of therapy whereas the concentration of HDL-cholesterol was slightly, but not significantly, increased while serum triglyceride was unchanged. Among these changes, only the reduction in the concentration of LDL-cholesterol reached significance in this small number of

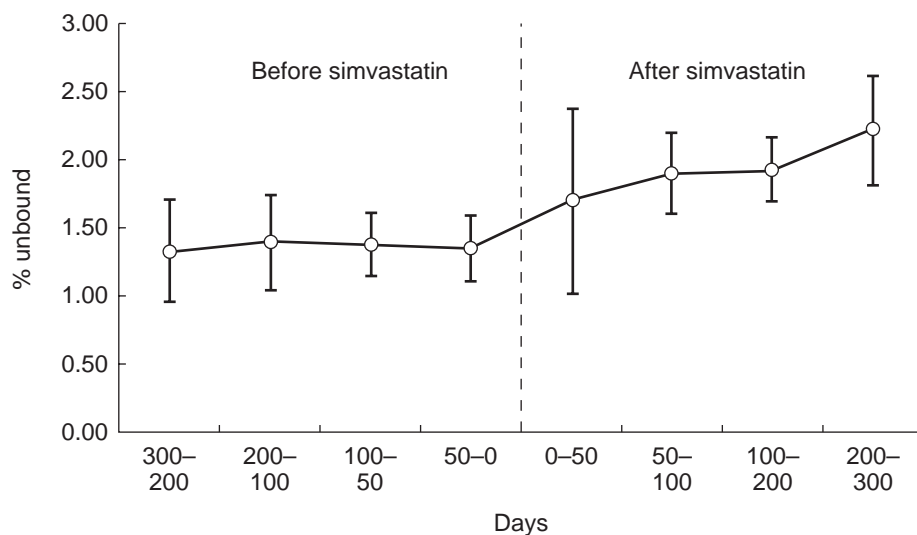


**Figure 1** Mean ( $\pm$ s.d.) of cyclosporine percentage unbound before and after simvastatin administration, study I, paired  $t$ -test. \* $P < 0.01$ .

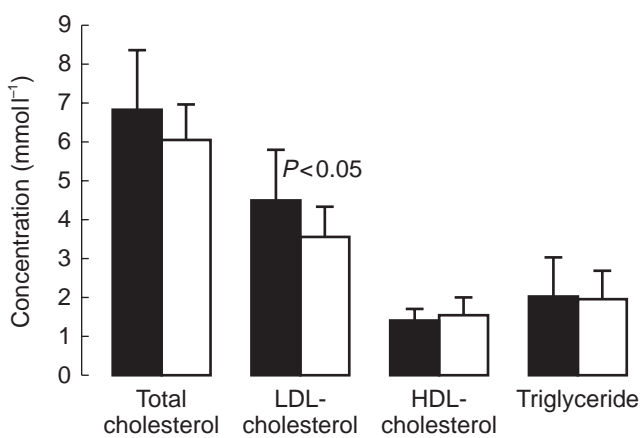
**Table 1** Composition of data sets.

Data set	Number of patients	Number of observations*	Number of observations per patient Mean (range)	Time post transplant (days)**	Simvastatin treatment (days)**
<i>Study I (fu)</i>					
Before simvastatin	12	31	2.5 (1-4)	$93 \pm 83$	$88 \pm 75$
After simvastatin	12	23	2 (1-4)	$230 \pm 111$	$123 \pm 97$
<i>Study II</i>					
Before simvastatin	32	172	6 (2-11)	$125 \pm 137$	$93 \pm 51$
After simvastatin	32	135	5 (1-12)	$271 \pm 211$	$53 \pm 56$

\*Each observation for study I is the value of cyclosporine fraction unbound and for study II is the oral dose rate of cyclosporine and the corresponding cyclosporine trough concentration at steady state; \*\*Data are presented as mean  $\pm$  s.d.



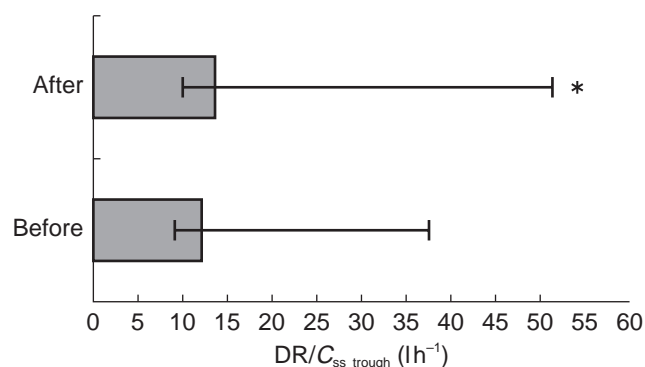
**Figure 2** Plot of cyclosporine percentage unbound in various time intervals before and after simvastatin administration in 12 heart transplant patients (study I), each data point represents mean percentage unbound and 95% confidence interval of the mean for that period of time.



**Figure 3** Concentrations of serum lipids in 12 heart transplant patients before (■) and after (□) simvastatin administration (study I). Data represent mean ( $\pm$ s.d.).

patients (paired *t*-test,  $P < 0.05$ ). In the population analysis of the individual Bayesian estimates of  $DR/C_{ss\text{trough}}$ , generated using P-PHARM, a number of potential covariables were examined for their effect including age, gender, weight, time since the last dose and co-administration of ketoconazole. Only the effect of ketoconazole on  $DR/C_{ss\text{trough}}$  was selected as a significant covariable.

Table 2 represents mean values of cyclosporine dosage and cyclosporine concentration for the transplant recipients included in the second study. The total cyclosporine concentration in whole blood was significantly lower after simvastatin administration as compared to before ( $P < 0.0001$ ) despite no significant differences in total and weight adjusted daily dose of cyclosporine. Table 3 displays the median of estimated  $DR/C_{ss\text{trough}}$  before and after



**Figure 4** Posterior Bayesian estimates of  $DR/C_{ss\text{trough}}$  before and after the administration of simvastatin in 32 heart transplant patient (study II), Data are median (interquartile range) of  $DR/C_{ss\text{trough}}$  for each group, \*Significantly greater than values obtained before simvastatin administration (Wilcoxon matched-pairs signed-ranks test,  $P < 0.05$ ).

simvastatin administration, the difference in the estimated  $DR/C_{ss\text{trough}}$  as a result of treatment and the ratio of  $DR/C_{ss\text{trough}}$  after and before simvastatin administration. The median and interquartile range for estimated  $DR/C_{ss\text{trough}}$  for patients before and after administration of lipid lowering agent is displayed in Figure 4. It is clearly shown (Table 3 and Figure 4) that there are wide inter-patient differences present in the estimated apparent clearance across the study population.

This study estimates that there is a median increase of  $18\text{ l h}^{-1}$  in cyclosporine  $DR/C_{ss\text{trough}}$  after simvastatin administration compared with before (Table 3). The mean of cyclosporine  $DR/C_{ss\text{trough}}$  was approximately  $24.5\text{ l h}^{-1}$  before and  $28.9\text{ l h}^{-1}$  after simvastatin administration. This

**Table 2** Cyclosporine dosage, weight adjusted dose and total concentration for 32 patients before and after simvastatin administration (study II).

Data set	Before simvastatin	After simvastatin	P value
Cyclosporine dose ( $\text{mg day}^{-1}$ )*	120 (80–350)	110 (60–300)	0.15
Weight adjusted dose ( $\text{mg kg}^{-1}\text{ day}^{-1}$ )*	1.6 (1–3.9)	1.4 (0.8–3.9)	0.10
Whole blood cyclosporine concentration ( $\mu\text{g l}^{-1}$ )*	334 (253–407)	235 (177–274)	0.0001

\*Values are expressed as median (interquartile range).

**Table 3** Posterior Bayesian estimates of cyclosporine DR/ $C_{ss\text{trough}}$  ( $1\text{h}^{-1}$ ).

	Number of patients	DR/ $C_{ss\text{trough}}$ ( $1\text{h}^{-1}$ )			
		Before simvastatin	After simvastatin	Difference (After–Before)	Ratio (After/Before)
Complete data set	32	12.1 (9.1–37.6)	13.7* (10.2–51.5)	18.2 (–3.1–42)	1.18 (0.96–1.42)
No ketoconazole	12	48.1 (35.2–56.3)	57.7** (41.5–67.3)	11.0 (–15.8–55)	1.11 (0.84–1.55)
With ketoconazole	20	10.1 (7.6–12.2)	10.9** (9.7–12.3)	21.9 (–2.92–42.13)	1.21 (0.97–1.42)

Values are expressed as median (interquartile range); \* $P < 0.05$ , \*\*NS.

increase was statistically significant even though number of patients examined were small ( $P < 0.05$ ). Values of posterior Bayesian estimates of DR/ $C_{ss\text{trough}}$  before and after simvastatin administration were not significantly different when data divided between two groups of transplant recipients who received ketoconazole as a cyclosporine sparing agent ( $P = 0.09$ , 20 patients) and those who did not ( $P = 0.24$ , 12 patients). The median difference in the DR/ $C_{ss\text{trough}}$  and the ratio of DR/ $C_{ss\text{trough}}$  after to before simvastatin administration were higher, to some extent, in the ketoconazole group indicating that the increase in cyclosporine clearance may happen to a greater extent in patients receiving ketoconazole (Table 3).

## Discussion

Lipid lowering therapy is commonly administered to post transplant patients to overcome hyperlipidaemia and to prevent coronary artery disease [8–10]. The HMG-CoA reductase inhibitors, including simvastatin, are among the preferred lipid lowering agents because they specifically reduce the concentration of LDL cholesterol. Considering the affinity of cyclosporine for plasma lipoproteins, it is expected that the alteration of lipoprotein concentration may influence cyclosporine fraction unbound or the percentage of cyclosporine associated with each lipoprotein fraction and consequently cyclosporine disposition may be affected. The increase in the cyclosporine fraction unbound associated with lipid lowering therapy has not previously been reported. The increase however, can be anticipated considering the inverse correlation of cyclosporine fraction unbound with the concentration of LDL and HDL cholesterol [4, 16].

In order to further investigate the potential effects of changes in cyclosporine binding during lipid lowering therapy on cyclosporine pharmacokinetics, we collected dose–concentration data in a second group of transplant recipients. The objectives of the present study were to investigate whether lipid lowering therapy will influence cyclosporine total blood concentration and clearance. To examine the clearance, a population pharmacokinetic approach was used since more detailed cyclosporine concentration time data were not available. Total cyclosporine concentration in whole blood and DR/ $C_{ss\text{trough}}$ , an estimation of apparent clearance, was compared for each individual patient on two different occasions, for a period of time before and after lipid lowering administration. The posterior Bayesian estimate of apparent clearance after administration of simvastatin was compared with similar estimates for a period of time before administration of

simvastatin for each patient in the study. To some extent this reduces the problem of inter-individual variability in cyclosporine pharmacokinetics including the effect of administration of metabolic inhibitors. This study found a moderate increase in the value of apparent clearance of cyclosporine in patients who were taking lipid lowering treatment. It is possible that this is related to the decrease in protein binding of cyclosporine in plasma observed in these patients. Cyclosporine is primarily metabolized by the liver [21] and its hepatic extraction ratio is considered to be low to intermediate [11]. Therefore the clearance of cyclosporine is expected to be affected by unbound fraction in plasma [12].

To consider the clinical significance of these findings for patients receiving cyclosporine therapy and lipid-lowering agents one has to consider whether total or unbound concentration of cyclosporine is pharmacologically active. Total concentration of cyclosporine in whole blood is routinely monitored following transplantation while routine measurement of unbound cyclosporine is restricted owing to the complexity of methods. For the majority of pharmaceutical agents, it is generally accepted that the unbound concentration in plasma is the active species at the receptor site [22] therefore it should correlate, more closely, with clinical outcomes. For cyclosporine, a number of researchers [23–25] have attempted to define the association between total cyclosporine concentration and its clinical outcomes, especially organ rejection. However, debate remains about the cyclosporine concentration range that will be effective against organ rejection without significant cyclosporine toxicity [24]. Lindholm and colleagues [26–28] measured the total concentration of cyclosporine and fraction unbound in plasma for renal transplant recipients and calculated unbound concentration in plasma. They observed that both total and unbound concentration as well as the fraction unbound were significantly lower at the time of rejection when compared with 1 week before rejection [28]. These workers concluded that routine measurement of unbound cyclosporine concentration is not recommended considering the complicated methodology involved.

In the light of these data it is possible to consider the clinical significance of the present findings. If the total concentration of cyclosporine is considered to be clinically important, following administration of simvastatin, dosage of cyclosporine has to be increased to maintain the optimal concentration with respect to time post transplantation. If the unbound concentration is proven to be clinically important, no dosage adjustment is required since for drugs with low extraction ratio, the free concentration will remain essentially constant as a result of reduced protein binding

despite the increase in clearance and reduced total concentration [29].

The difference in the values of cyclosporine clearance before and after simvastatin administration was slightly greater for the subgroup of patients receiving ketoconazole as compared with the others. It has been speculated that ketoconazole may reduce cyclosporine metabolism by inhibition of gastrointestinal P450 enzymes [30] therefore, for patients receiving ketoconazole, hepatic metabolism would become the major route of elimination. Considering the influence of fraction unbound on hepatic metabolism, the greater difference in the clearance of cyclosporine observed in the ketoconazole patients may be explained.

In the light of the present findings it should be borne in mind that since the cyclosporine clearance may be increased following co-administration of lipid lowering agents, monitoring of cyclosporine total blood concentration and careful assessment of transplant status is advisable whenever lipid lowering agents are instituted in patients on cyclosporine.

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