

# Blood and Plasma Pharmacokinetics of Ciclosporin in Diabetic Kidney Transplant Recipients

Anisha E. Mendonza,<sup>1</sup> Reginald Y. Gohh<sup>2</sup> and Fatemeh Akhlaghi<sup>1</sup>

<sup>1</sup> Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, Rhode Island, USA

<sup>2</sup> Division of Organ Transplantation, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

## Abstract

**Background and objectives:** Long-term diabetes mellitus may affect the absorption, distribution and metabolism of immunosuppressive agents used after organ transplantation. The aims of this study were to characterize ciclosporin pharmacokinetics in blood and plasma and to compare the ciclosporin unbound concentration and the blood : plasma concentration (B : P) ratio in diabetic kidney transplant recipients.

**Patients and methods:** Ciclosporin 12-hour steady-state pharmacokinetics were studied in eight diabetic and nine nondiabetic patients. Ciclosporin concentrations in whole blood and in plasma were measured using liquid chromatography-tandem mass spectrometry, and the ciclosporin fraction unbound ( $f_u$ ) was determined by an equilibrium dialysis method utilizing [<sup>3</sup>H]ciclosporin as a tracer. Oral absorption of paracetamol (acetaminophen) was used as a marker for gastric emptying.

**Results:** In diabetic patients, the time to the peak blood ciclosporin concentration at steady state ( $t_{max,ss}$ ) was prolonged (128 minutes vs 93 minutes in nondiabetic patients,  $p < 0.01$ ) and, on average, the paracetamol  $t_{max}$  was prolonged by 30 minutes. The whole-blood dose-normalized area under the concentration-time curve from 0 to 12 hours ( $AUC_{12}$ ) was marginally lower in diabetic patients ( $p = 0.09$ ) and the plasma  $AUC_{12}$  was significantly lower ( $p = 0.03$ ). The ciclosporin  $f_u$  was numerically higher in diabetic patients ( $1.20 \pm 0.65\%$  vs  $0.72 \pm 0.28\%$  in nondiabetic patients,  $p = 0.066$ ); however, the unbound concentration values were essentially similar in the two groups ( $0.58 \pm 0.76 \mu\text{g/L}$  in diabetic patients and  $0.52 \pm 0.48 \mu\text{g/L}$  in nondiabetic patients;  $p = 0.59$ ). No difference was observed in the ciclosporin B : P ratio between the two groups.

**Conclusion:** This study indicates that diabetes delays ciclosporin absorption, reduces ciclosporin exposure and increases the ciclosporin  $f_u$  but not the pharmacologically active unbound concentration.

## Background

The immunosuppressive agent ciclosporin is used in combination therapy with mycophenolic acid and prednisone for prevention of organ transplant rejection.<sup>[1]</sup> Ciclosporin has a narrow therapeutic index and exhibits variable and unpredictable pharmacokinetic properties, necessitating routine therapeutic drug monitoring.<sup>[2]</sup> Factors contributing to this variability include age, the time post-transplant, interacting drugs, the extent of presystemic elimination by cytochrome P450 (CYP) 3A or the efflux transporter P-glycoprotein (multidrug resistance transporter 1) in the gastrointestinal tract, and the concentration of serum lipoproteins.<sup>[3-6]</sup>

Trough concentrations ( $C_{trough}$ ) of ciclosporin in whole blood are routinely measured after transplantation as a guide to dose

adjustment. However, patients may experience episodes of rejection or nephrotoxicity despite  $C_{trough}$  values within the desirable range.<sup>[2,7,8]</sup> In blood, ciclosporin is highly bound to blood cells and plasma proteins, leaving an unbound fraction ( $f_u$ ) of ~2%.<sup>[3]</sup> It is generally accepted that both the pharmacokinetic and pharmacodynamic properties of drugs are related to unbound drug concentrations rather than total concentrations.<sup>[9]</sup> This concept is particularly true for highly protein-bound drugs with an  $f_u$  of less than 30–40%.<sup>[9,10]</sup>

In kidney transplant recipients, Lindholm<sup>[11]</sup> observed a significant decline in ciclosporin  $f_u$  in the week preceding an allograft rejection. A study by our research group in heart transplant recipients showed that the ciclosporin  $f_u$  was significantly lower in hyperlipidaemic patients and that this was associated with an

increase in the frequency of heart allograft rejections.<sup>[12]</sup> Understanding of the interrelationship between total and unbound concentrations of ciclosporin in patients with diseases, such as diabetes mellitus, that potentially affect drug disposition is therefore important in improving dose optimization of ciclosporin.<sup>[13]</sup>

According to the statistics published by the American Diabetes Association, approximately 7% or 20.8 million of the US population were diabetic in 2005. The data from the US Centers for Disease Control and Prevention also indicate that diabetes was the aetiology of end-stage kidney disease in 44% of cases.<sup>[14]</sup> Correspondingly, 30–40% of all kidney transplant recipients in the US are diabetic at the time of transplantation and an additional 10–15% develop diabetes after transplantation, a condition that is known as post-transplant diabetes.<sup>[15]</sup>

Long-term diabetes affects the autonomic nervous system and can consequently alter the motility of the gastric muscles, resulting in delayed gastric emptying.<sup>[16]</sup> The delay in gastric emptying can theoretically prolong the oral absorption of drugs, thereby influencing pharmacokinetic parameters such as the maximum steady-state blood/plasma concentration ( $C_{\max,ss}$ ), the time to reach the  $C_{\max,ss}$  ( $t_{\max,ss}$ ), or the area under the blood/plasma concentration-time curve (AUC). Hyperglycaemia modifies the micro-environmental condition of the type 2 binding site of albumin, resulting in reduced binding capacity of albumin-bound drugs.<sup>[17,18]</sup> In addition, increases in the concentrations of triglyceride and free fatty acids in plasma may influence the plasma protein binding of drugs.<sup>[17]</sup>

Very little is known about the effect of diabetes on human drug-metabolizing enzymes or transporters, although both insulin and glucagon may regulate these systems.<sup>[19]</sup> In addition, conditions such as non-alcoholic steatohepatitis (non-alcoholic fatty liver disease) that is present in a large number of patients with type 2 diabetes can profoundly influence the capacity of the liver to metabolize xenobiotics.<sup>[20]</sup> Furthermore, in streptozotocin-induced diabetic rats, ciclosporin clearance was reduced by 127%, indicating that overt diabetes may profoundly reduce ciclosporin metabolism.<sup>[21]</sup> Insulin injections restored ciclosporin clearance; however, it was still 18% lower than normal in diabetic rats. In streptozotocin-induced diabetic non-human primates undergoing islet transplantation, ciclosporin generated severe renal dysfunction in some animals.<sup>[22]</sup>

Apart from brief reports on ciclosporin  $C_{\text{trough}}$  values<sup>[23]</sup> or ciclosporin pharmacokinetics in kidney-pancreas transplant recipients with gastroparesis,<sup>[24]</sup> the disposition of ciclosporin has not been systematically investigated in diabetic transplant recipients. The objectives of this study were to characterize the pharmacokinetics of ciclosporin in blood and plasma and to estimate its  $f_u$  in

diabetic stable kidney transplant recipients in comparison with demographically matched nondiabetic patients.

## Methods

The study protocol was approved by the Institutional Review Board of Rhode Island Hospital (Providence, RI, USA). Patients were recruited by kidney transplant physicians and, before the study, all procedures were explained to them verbally and their signed informed consent was obtained.

### Study Population

The study population consisted of eight diabetic and nine nondiabetic stable kidney transplant recipients. Diabetic patients were broadly matched with nondiabetic patients based on sex, age, ethnicity, time post-transplant, mycophenolic acid dose and kidney function. All patients were on steady-state treatment with ciclosporin, with doses adjusted on the basis of routinely measured  $C_{\text{trough}}$  values in whole blood by the enzyme-multiplied immunoassay technique. Dosage adjustment was aimed for a ciclosporin blood concentration of 300–400  $\mu\text{g/L}$  in the first year post-transplant and 75–150  $\mu\text{g/L}$  thereafter. Patients who were pregnant or nursing and those who had received a pancreas transplant were excluded. The immunosuppressive protocol included triple therapy with oral ciclosporin (Neoral<sup>®</sup>;<sup>1</sup> Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA [ $n = 14$ ] or its bioequivalent and interchangeable generic form, Gengraf<sup>®</sup>; Abbott Laboratories, North Chicago, IL, USA [ $n = 3$ ]),<sup>[25]</sup> mycophenolate mofetil (Cellcept<sup>®</sup>; Roche Laboratories Inc., Nutley, NJ, USA [ $n = 11$ ] or Myfortic<sup>®</sup>; Novartis Pharmaceuticals Corporation [ $n = 6$ ]) and prednisone.

In addition to the immunosuppressive agents and medications used to treat diabetes (seven patients on short-, intermediate- or long-acting insulin, one patient on an oral antihyperglycaemic agent), the diabetic patients received 14 other medications compared with ten other medications in the nondiabetic group. The most frequently prescribed types of medication in the two groups were ACE inhibitors (five diabetic and three nondiabetic patients), sulfamethoxazole/trimethoprim (three diabetic and three nondiabetic patients),  $\beta$ -adrenoceptor antagonists (six diabetic and five nondiabetic patients), calcium channel antagonists, amlodipine or nifedipine (four diabetic and four nondiabetic patients), loop diuretics (five diabetic and two nondiabetic patients), proton pump inhibitors (four diabetic and three nondiabetic patients) and HMG-CoA reductase inhibitors (seven diabetic and six nondiabetic patients). At our centre, the azole antifungal agent ketoconazole is used routinely as a ciclosporin-sparing agent in patients who can tolerate it. In this study, six diabetic patients and nine nondiabetic

1 The use of trade names is for product identification purposes only and does not imply endorsement.

patients were receiving ketoconazole and ciclosporin concomitantly.

### Study Design

Patients were instructed to fast from the night before the study and to arrive at the hospital at 7:00am. On the study day, patients underwent a physical examination, which included measurement of the blood pressure, bodyweight and height, and urinalysis, after which a baseline blood sample (at 0 hours) was drawn via an intravenous catheter. The patients then took their regular morning medications at approximately 8:00am with 250 mL of water and were given paracetamol (acetaminophen) 1000 mg (Concentrated Tylenol® Infants' Drops; McNeil Consumer and Specialty Pharmaceuticals, Washington, PA, USA), to serve as a marker for the gastric emptying rate. To prevent hypoglycaemia, patients were allowed to consume a fruit-juice-based nutritional drink (Boost Breeze; Novartis Nutrition Corporation, Basel, Switzerland) at any time. Nevertheless, patients remained fasted for 2 hours after oral medications were given. All patients were given standardized diabetic hospital meals (with a total intake of 2000 Kcal [8372 kJ] per day) at approximately 10:00am, 1:00pm and 7:00pm. Blood was collected into Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing EDTA as an anticoagulant at 0.25, 0.5, 1, 1.5, 2, 3, 5, 7, 9, 10 and 12 hours post-dose. Biochemical indices, including creatinine, glucose, liver function tests, serum lipids and glycosylated haemoglobin (HbA<sub>1c</sub>), were measured by an accredited clinical laboratory (Labcorp®). The concentration of glycated albumin was measured in plasma using ELISA (Glycaben®; Exocell, Inc., Philadelphia, PA, USA).

### Analysis of Drug Concentrations

All assays were performed at the Clinical Pharmacokinetics Research Laboratory, University of Rhode Island. For determination of ciclosporin total or unbound concentrations in plasma, whole blood was warmed in a water bath to 37°C and centrifuged at 1500 × *g* and 37°C using a SORVALL Legend RT temperature-controlled centrifuge (Kendro Lab Products, Newtown, CT, USA). The temperature was maintained at 37°C because the blood : plasma (B : P) concentration ratio of ciclosporin varies with temperature.<sup>[26]</sup> All blood and plasma samples were kept frozen at -80°C until analysis. Plasma concentrations of paracetamol were measured, as described previously, by high-performance liquid chromatography (HPLC) with UV detection.<sup>[27]</sup> Ciclosporin concentrations in whole blood or in plasma were measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, as described below. Both assays were validated on the basis of US FDA guidelines.<sup>[28]</sup> The *f<sub>u</sub>* of ciclosporin was measured as described using equilibrium dialysis.

### Analysis of Ciclosporin Concentration in Blood or Plasma

The assay method that was used is a modification of a previously reported assay for analysis of ciclosporin in saliva.<sup>[29]</sup> All solvents were HPLC grade and chemicals were analytical grade.

#### Extraction

Patient blood or plasma samples and corresponding calibrators were thawed at room temperature, and an aliquot (500 µL) was pipetted into the precipitating reagent (1 mL). The precipitating reagent consisted of 125 µg/L internal standard (ciclosporin C) in acetonitrile. Samples were vortex-mixed for 1 minute and centrifuged at 860 × *g* and 4°C for 5 minutes. The supernatants were then subjected to solid-phase extraction cartridges (Sep-Pak C18, 200 mg, 3 mL capacity) [Waters Corp., Milford, MA, USA] that were preconditioned with methanol (6 mL) and water (6 mL). On passage of the supernatant, the cartridges were washed with 6 mL of de-ionized water, followed by 3 mL of 50/50 (v/v) methanol/water and 2 mL of heptane. The cartridges were dried for 15 minutes under full vacuum, and the analytes were eluted with 2.5 mL of isopropanol and heptane (50/50 v/v) and dried in a centrifugal evaporator (Model SPD1010 Speed Vac® System; ThermoSavant, Holbrook, NY, USA) at 60°C for approximately 50 minutes. The residues were then reconstituted in 250 µL of absolute methanol, and 100 µL was injected onto the LC-MS/MS system.

#### Instrumentation

Quantification of the ciclosporin concentrations in whole blood or in plasma was carried out using an LC-MS/MS system consisting of an autosampler and micropumps (Perkin Elmer, Norwalk, CT, USA) coupled with an ABI-Sciex 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA). The analytical column was an AquaPerfect C18 (150 × 3 mm, 5 µm) reversed phase column (MZ Analysentechnik GmbH, Mainz, Germany). Mobile-phase composition consisted of methanol and 30 mmol/L ammonium acetate (80/20 v/v) at a flow rate of 0.25 mL/min for 1 minute, which was switched to 97/3 v/v at 0.35 mL/min for 4.5 minutes and then switched back to 80/20 composition at 0.25 mL/min for the last 1.5 minutes. Analytes were detected in positive ionization mode using the mass transitions of *m/z* 1219.6→1202.6 for ciclosporin and *m/z* 1235.3→1218.3 for internal standard.

#### Validation

The lower limit of quantification for ciclosporin was 10 µg/L in blood or plasma, and the limit of detection was 2 µg/L. The calibration curve was constructed by spiking drug-free blood or plasma with ciclosporin at varying concentrations (10, 25, 50, 100, 250, 500, 1000 and 2500 µg/L). The absolute recovery for ciclosporin and internal standard ranged between 90–96% and 91–95%, respectively. The absolute recovery was measured by comparing the peak area for the analyte extracted from the matrix

(blood or plasma) with the peak area of the analyte directly injected in the mobile phase. The intra- and inter-day coefficient of variation (CV) for the quality controls at 25 µg/L, 200 µg/L and 1500 µg/L concentrations ranged between 2.0–2.1% and 4.9–10.6%, respectively. The inaccuracy for the method calculated based on the CV for quality control samples was  $5.3 \pm 1.6\%$  for 25 µg/L,  $4.7 \pm 0.9\%$  for 200 µg/L and  $4.8 \pm 2.5\%$  for 1500 µg/L. The average calibration curve equation was (equation 1):

$$Y = 0.0092X - 0.1215 \quad (r^2 = 0.98) \quad (\text{Eq. 1})$$

where X is the ciclosporin concentration and Y is the peak area ratio.

#### Determination of Ciclosporin Unbound Fraction

The ciclosporin  $f_u$  in plasma was estimated using an equilibrium dialysis method as previously described,<sup>[30]</sup> utilizing HPLC purified [<sup>3</sup>H]ciclosporin to eliminate radiolabelled impurities and equilibrium dialysis chambers custom-built from medical-grade stainless steel. Briefly, a 1 mL methanol solution of ciclosporin containing 29.4 µCi (equivalent to 3.9 µg [<sup>3</sup>H]ciclosporin) and 20.6 µg non-radiolabelled ciclosporin was prepared. A 20 µL aliquot of this solution was injected onto an AquaPerfect analytical column maintained at 65°C. The mobile phase consisted of 76/24 v/v acetonitrile and de-ionized water. The retention times for ciclosporin C and ciclosporin A were approximately 1.8 and 2.4 minutes, respectively, indicating that the chromatographic method was suitable for resolving compounds with structures similar to ciclosporin. HPLC fractions corresponding to the ciclosporin peak detected by the UV detector were collected into silanized culture tubes and dried at 45°C under a fume hood using a dry-heat block, and the residues were reconstituted in isopropanol. The reconstituted residues were utilized to spike patient plasma samples, as described below.

#### Equilibrium Dialysis Method

Plasma samples from each patient, that were near the maximum ciclosporin concentration, were thawed after being frozen at -70°C and then pooled to obtain a total volume of 3 mL. The pooled samples enabled determination of the  $f_u$  in triplicates (0.8 mL per sample) for each subject. These samples were spiked with purified [<sup>3</sup>H]ciclosporin in isopropanol (less than 0.2% v/v isopropanol in plasma) to give an approximate activity of 200 000 disintegrations per min/mL (dpm/mL). Plasma was dialysed against isotonic phosphate buffer using silanized stainless-steel dialysis cells (each chamber had a capacity of 1 mL), constructed by Fitzwater Engineering Corporation, Scituate, RI, USA) in a 37°C water bath. Cellulose dialysis membranes with a molecular weight cut-off of 12 000–14 000 (2 Spectra/Por® RC, Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) and Dia-

Norm equilibrium dialyser apparatus (DiaNorm-Geräte, Munich, Germany) were used. After an equilibration time of 18 hours, plasma and buffer were sampled simultaneously using silanized stainless-steel tubing. A 0.1 mL volume of plasma or buffer was pipetted into the scintillation vials containing 3 mL of scintillant (Emulsifier Safe, Perkin Elmer, Boston, MA, USA). The pipette tips were rinsed with water (0.1 mL) and methanol (0.1 mL), and all of the rinsings were pooled with the sample. The radioisotopic activity was measured in dpm using a liquid scintillation counter (LS 6500 Multipurpose Scintillation Counter, Beckman Coulter, Fullerton, CA, USA).

The concentration of total plasma proteins ( $C_{\text{prot}}$ ) before and after dialysis was measured using a bicinchoninic acid protein assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA), and the volume of plasma at equilibrium ( $V_{\text{p, equil}}$ ) [volume shift] was calculated using equation 2:

$$V_{\text{p, equil}} = \frac{C_{\text{prot, initial}}}{C_{\text{prot, after equil}}} \cdot V_{\text{p, before equil}} \quad (\text{Eq. 2})$$

The ciclosporin  $f_u$  (and expressed as a percentage, % $f_u$ ) was then calculated using equation 3:

$$f_u = \frac{C_{\text{buffer, equil}}}{(C_{\text{p, equil}} - C_{\text{buffer, equil}}) \times V_{\text{p, equil}} + C_{\text{buffer, equil}}} \quad (\text{Eq. 3})$$

where  $C_p$  is the concentration of ciclosporin in plasma and  $C_{\text{buffer}}$  is the concentration of ciclosporin in the buffer. The unbound concentration of ciclosporin ( $C_u$ ) was calculated for each time-point according to equation 4:

$$C_u = f_u \times C_p \quad (\text{Eq. 4})$$

#### Pharmacokinetic Analysis

Pharmacokinetic parameters from various concentration-time data (whole blood or plasma) were calculated using WinNonlin version 5.0.1 software (Pharsight Corporation, Mountain View, CA, USA). A noncompartmental model with extravascular input was used to obtain estimates of the  $C_{\text{max, ss}}$ ,  $t_{\text{max, ss}}$ , elimination half-life ( $t_{1/2}$ ), area under the concentration-time curve (over 12 hours) [AUC<sub>12</sub>] calculated using the linear trapezoidal rule, oral clearance at steady-state ( $CL/F_{\text{ss}}$ ) and volume of distribution at steady-state after oral administration ( $V_{\text{ss}}/F$ ). The ciclosporin B : P ratio, a measure of the affinity of blood cells for the drug, was calculated as the concentration in whole blood/plasma. Moreover, morning and evening  $C_{\text{trough}}$  values ( $C_0$  and  $C_{12}$ , respectively) and the %fluctuation were compared between the two groups. The %fluctuation was calculated using equation 5:

$$\% \text{fluctuation} = \frac{C_{\max,ss} - C_{\min,ss}}{C_{\text{avg}}} \times 100 \quad (\text{Eq. 5})$$

where  $C_{\text{avg}}$  (average ciclosporin concentration) is  $AUC_{12/12}$ . Dose-normalized and non-dose-normalized values of the  $C_{\max,ss}$ , morning and evening  $C_{\text{trough}}$  and  $AUC_{12}$  were calculated by dividing the pharmacokinetic parameter by the ciclosporin dose in micrograms multiplied by 1000.

### Statistical Analysis

All statistical comparisons were performed using SPSS version 15 software (SPSS Inc, Chicago, IL, USA). The Kolmogorov-Smirnov test with the Lilliefors correction was used to detect non-Gaussian distribution. Unless otherwise stated, all data were expressed as the mean  $\pm$  SD if normally distributed or the median and interquartile range if non-normally distributed. An independent samples t-test was used to compare normally distributed data and a Mann-Whitney U test was used for comparisons of non-normally distributed data. Pearson or Spearman correlation coefficients were computed for comparisons. The significance level was based at 0.05.

### Results

Demographic characteristics, biochemical indices and immunosuppressant doses are given in table I. The aetiology of end-stage kidney disease was diabetes ( $n = 8$ ) in diabetic patients and IgA nephropathy ( $n = 3$ ), membranous glomerulonephritis ( $n = 3$ ), crescentic glomerulosclerosis ( $n = 1$ ) and hypertension ( $n = 2$ ) in nondiabetic patients. Both groups had comparable biochemistry except for higher  $HbA_{1c}$  and glucose levels in diabetic patients (table I). The whole-blood ciclosporin pharmacokinetic parameters were calculated for eight diabetic and nine nondiabetic patients. However, because of lack of sufficient plasma to measure ciclosporin concentration in some patients, plasma pharmacokinetic parameters were estimated for only six diabetic and seven nondiabetic patients.

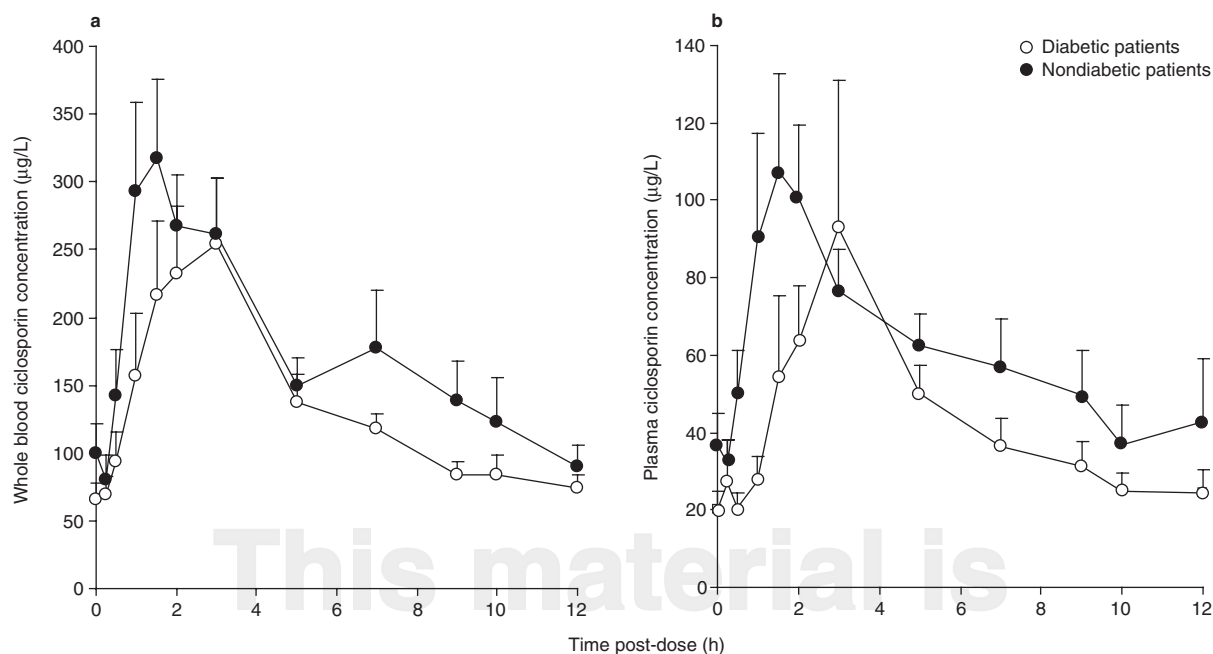
Figure 1 represents the mean concentration-time profiles of total ciclosporin in whole blood (figure 1a) and in plasma (figure 1b). In addition, ciclosporin pharmacokinetic parameters are compared between diabetic and nondiabetic patients based on whole-blood concentrations in table II and plasma concentrations in table III. Because the ciclosporin morning dose was numerically higher ( $p = 0.19$ ) in diabetic patients (table I), dose-normalized pharmacokinetic parameters are also presented in tables II and III.

**Table I.** Demographic characteristics, biochemical indices and immunosuppressant dosages in diabetic and nondiabetic patients<sup>a</sup>

Parameter	Diabetic patients (n = 8)	Nondiabetic patients (n = 9)
Sex (male/female) [n]	7/1	8/1
Age (y)	51 (45–59)	56 (45–63)
Bodyweight (kg)	93 (73–121)	83 (74–105)
Time post-transplant (mo)	49 (37–70)	59 (30–70)
Ciclosporin morning dose (mg/day)	50 (25–75)	25 (25–50)
Ciclosporin daily dose (mg)	87 (50–219)	50 (50–88)
Prednisone dose (mg/day)	5.0 (5.0–6.8)	5.0 (5.0–7.5)
Mycophenolic acid dose (mg/day)		
Cellcept® (n = 11)	1000 (500–1000)	500 (500–1000)
Myfortic® (n = 6)	540 (360–720)	720 (450–720)
Blood glucose (mg/dL)	128 (99–194)	80 (75–87)*
HbA <sub>1c</sub> (%)	7.5 (7.3–9.5)	5.4 (5.3–5.5)**
Total albumin (mg/dL)	4.2 (4.0–4.5)	4.3 (4.2–4.4)
Glycated albumin (mg/dL)	1.3 (0.9–1.6)	1.1 (0.7–1.5)
Total cholesterol (mg/dL)	179 (139–215)	174 (160–208)
LDL cholesterol (mg/dL)	89 (69–131)	99 (56–108)
HDL cholesterol (mg/dL)	52 (50–64)	55 (42–67)
Triglyceride (mg/dL)	112 (77–137)	117 (82–255)
Creatinine	1.6 (1.4–1.7)	1.6 (1.3–1.9)

<sup>a</sup> Values are expressed as median (interquartile range), unless specified otherwise.

**HbA<sub>1c</sub>** = glycosylated haemoglobin; **HDL** = high-density lipoprotein; **LDL** = low-density lipoprotein; \*  $p < 0.01$ , \*\*  $p < 0.001$  vs diabetic patients.



**Fig. 1.** Mean (standard error of the mean) concentration-time profiles of ciclosporin (a) in whole blood and (b) in plasma from diabetic and nondiabetic stable kidney transplant recipients.

#### Ciclosporin Time to Reach Peak Concentration and Gastric Emptying Time

The delay in gastric emptying was evident from the paracetamol concentration-time profiles (data not shown). On average, the paracetamol  $t_{\max}$  was prolonged in diabetic patients by ~30 minutes ( $67 \pm 36$  minutes vs  $41 \pm 35$  minutes in nondiabetic patients,  $p = 0.12$ ) and the  $C_{\max}$  was lower ( $9.9 \pm 3.8$  µg/L vs  $13.9 \pm 4.2$  µg/L in nondiabetic patients,  $p = 0.07$ ). However, exposure to parace-

tamol over 5 hours ( $AUC_5$ ) was similar ( $p = 0.87$ ) in the two groups.

Figure 1 clearly shows a pronounced prolongation of the ciclosporin  $t_{\max,ss}$  in diabetic patients. Corresponding to the paracetamol concentration-time profile, the ciclosporin  $t_{\max,ss}$  was significantly prolonged by 27% based on whole-blood data (table II) and by 36% based on plasma data (table III). The correlation coefficients between the ciclosporin and acetaminophen  $t_{\max,ss}$

**Table II.** Whole-blood pharmacokinetic parameters of ciclosporin in diabetic and nondiabetic stable kidney transplant patients<sup>a</sup>

Parameter	Non-dose-normalized			Dose-normalized		
	diabetic patients (n = 8)	nondiabetic patients (n = 9)	p-value	diabetic patients (n = 8)	nondiabetic patients (n = 9)	p-value
$t_{\max,ss}$ (min)	128 (107–184)	93 (71–112)	0.01			
$C_{\max,ss}$ (µg/L)	240.2 (187.1–400.5)	294 (195.2–580.7)	0.38	5.8 (1.7–8.3)	10.5 (7.0–13.2)	0.10
$AUC_{12}$ (µg • h/L)	1514 (1142–2055)	2157 (1314–2537)	0.18	36 (13–50)	54 (44–86)	0.09
Morning $C_{trough}$ (µg/L)	54.4 (50.1–98.3)	74.1 (53.5–151.8)	0.29	1.5 (0.9–2.2)	2.8 (1.8–5.9)	0.05
Evening $C_{trough}$ (µg/L)	64.4 (54.0–96.4)	71.5 (60.5–96.4)	0.50	1.3 (0.7–3.1)	2.0 (1.6–3.5)	0.08
$t_{1/2}$ (h)	5.5 (3.5–8.3)	6.6 (3.9–8.0)	0.94			
$CL/F_{ss}$ (L/h/kg)	0.28 (0.15–0.80)	0.19 (0.14–0.29)	0.29			
$V_{ss}/F$ (L/kg)	2.1 (1.4–9.3)	1.3 (0.8–3.2)	0.21			
Fluctuation (%)	171 (146–191)	141 (107–243)	0.92			

a Values are expressed as median (interquartile range).

**AUC<sub>12</sub>** = area under the plasma concentration-time curve from 0 to 12 h; **CL/F<sub>ss</sub>** = apparent oral clearance at steady state; **C<sub>max,ss</sub>** = peak plasma concentration at steady state; **C<sub>trough</sub>** = trough plasma concentration; **t<sub>1/2</sub>** = elimination half-life; **t<sub>max,ss</sub>** = time to reach  $C_{\max,ss}$ ; **V<sub>ss</sub>/F** = apparent volume of distribution at steady state after oral administration.

**Table III.** Plasma pharmacokinetic parameters of ciclosporin in diabetic and nondiabetic stable kidney transplant patients<sup>a</sup>

Parameter	Non-dose-normalized			Dose-normalized		
	diabetic patients (n = 6)	nondiabetic patients (n = 7)	p-value	diabetic patients (n = 6)	nondiabetic patients (n = 7)	p-value
$t_{\max,ss}$ (min)	187 (168–238)	120 (91–185)	0.09			
$C_{\max,ss}$ ( $\mu\text{g/L}$ )	69.7 (40.4–159.4)	150 (54.5–176.4)	0.32	2.3 (0.6–2.7)	3.0 (2.2–4.7)	0.09
$AUC_{12}$ ( $\mu\text{g} \cdot \text{h/L}$ )	468 (338–793)	884 (424–957)	0.19	11 (5–17)	18 (17–32)	0.03
Morning $C_{\text{trough}}$ ( $\mu\text{g/L}$ )	21.4 (15.3–32.4)	36.0 (12.7–42.9)	0.47	0.4 (0.2–1.1)	0.7 (0.4–1.7)	0.20
Evening $C_{\text{trough}}$ ( $\mu\text{g/L}$ )	18.6 (10.0–27.4)	30.1 (16.7–61.2)	0.12	0.4 (0.3–0.5)	1.1 (0.6–2.5)	0.007
$t_{1/2}$ (h)	6.7 (3.4–10.7)	5.1 (3.5–8.0)	0.44			
$CL/F_{ss}$ (L/h/kg)	1.2 (0.6–1.9)	0.5 (0.4–0.7)	0.07			
$V_{ss}/F$ (L/kg)	7.7 (3.9–18.0)	3.5 (2.8–5.1)	0.06			
Fluctuation (%)	158 (115–231)	154 (118–81)	0.67			

a Values are expressed as median (interquartile range).

**AUC<sub>12</sub>** = area under the plasma concentration-time curve from 0 to 12 h; **CL/F<sub>ss</sub>** = apparent oral clearance at steady state; **C<sub>max,ss</sub>** = peak plasma concentration at steady state; **C<sub>trough</sub>** = trough plasma concentration; **t<sub>1/2</sub>** = elimination half-life; **t<sub>max,ss</sub>** = time to reach C<sub>max,ss</sub>; **V<sub>ss</sub>/F** = apparent volume of distribution at steady state after oral administration.

were 0.29 for the  $t_{\max,ss}$  based on whole-blood data ( $p = 0.27$ ) and 0.52 based on plasma data ( $p = 0.07$ ).

#### Extent of Exposure to Ciclosporin

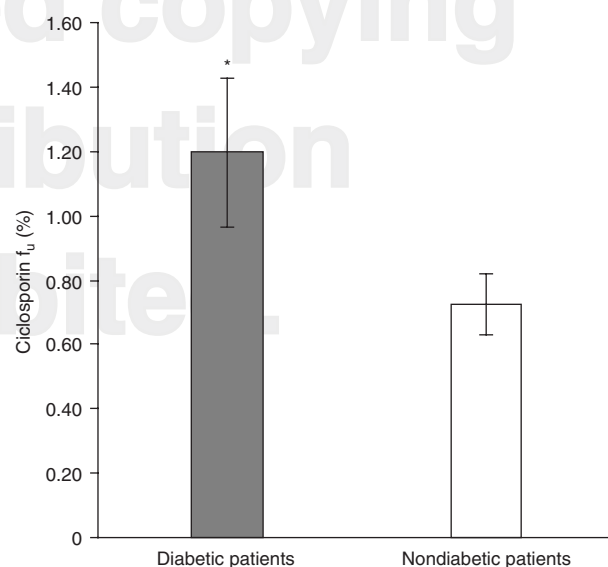
The ciclosporin  $C_{\max,ss}$ ,  $AUC_{12}$  and morning and evening  $C_{\text{trough}}$  values ( $C_0$  and  $C_{12}$ , respectively) are reported in table II for whole blood and in table III for plasma. All of these parameters were numerically lower in diabetic patients in both whole blood and plasma, especially when dose-normalized values were considered. More specifically, the extent of exposure ( $AUC_{12}$ ) was reduced in diabetic patients by 30% in whole blood and 47% in plasma (tables II and III). The ciclosporin  $AUC_{12}$ , calculated on the basis of dose-normalized plasma concentrations, was significantly lower in diabetic patients than in nondiabetic patients ( $p = 0.03$ ). The  $CL/F_{ss}$  and  $V_{ss}/F$  were higher in diabetic patients than in nondiabetic patients, but the difference did not reach statistical significance. The  $t_{1/2}$  values were comparable between the two groups (tables II and III). Moreover, the ciclosporin %fluctuation was similar in diabetic and nondiabetic patients.

Coadministration of ciclosporin and ketoconazole results in a marked increase in the ciclosporin concentration due to inhibition of hepatic and intestinal CYP3A activity.<sup>[31]</sup> Of 19 patients in this study, all but two were taking ketoconazole routinely with ciclosporin. Exclusion of those two patients did not influence the estimated pharmacokinetic parameters.

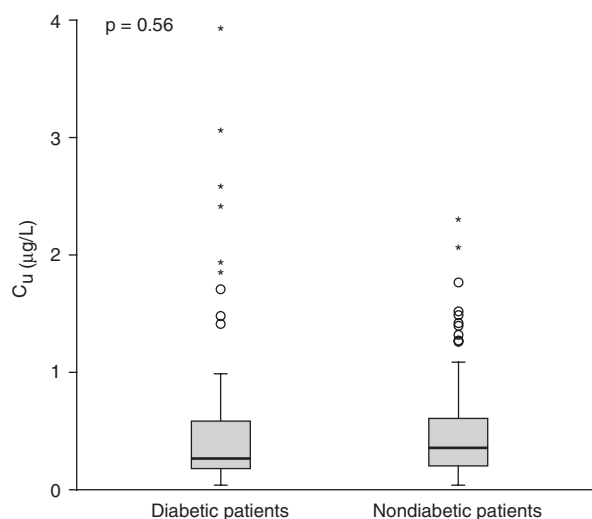
#### Ciclosporin Unbound Fraction and Concentration

The ciclosporin  $f_u$  was determined in triplicate in seventeen patients. The  $f_u$  was  $1.20 \pm 0.65\%$  (range 0.53–2.50%) in diabetic patients and  $0.72 \pm 0.28\%$  (range 0.43–1.18%) in nondiabetic

patients ( $p = 0.06$ ) [figure 2]. The values of the ciclosporin  $f_u$  were positively associated with HbA<sub>1c</sub> levels ( $r = 0.54$ ,  $p = 0.02$ ), indicating that plasma protein binding of ciclosporin may be affected by the degree of glucose control. No other significant associations were evident between the  $f_u$  and demographic or biochemical characteristics, including age, bodyweight, time post-transplant and the concentrations of plasma lipoproteins, cholesterol or albumin. Box plots summarizing the ciclosporin unbound concentration values in diabetic and nondiabetic patients are shown in figure 3. With the exception of a few outliers in the



**Fig. 2.** Ciclosporin fraction unbound ( $f_u$ ) in diabetic and nondiabetic stable kidney transplant recipients. The error bars denote the standard error of the mean. \*  $p = 0.066$  vs nondiabetic patients.



**Fig. 3.** Box plot of unbound cyclosporin concentrations ( $C_u$ ) in plasma from diabetic and nondiabetic kidney transplant recipients. The  $C_u$  was calculated using the equation  $C_u = f_u \times C_p$ , where  $f_u$  is the unbound fraction and  $C_p$  is the cyclosporin concentration in plasma. The central line in the box represents the median value, the lower and upper lines represent the 25th and 75th percentiles, respectively, the whiskers represent values  $<1.5$  times the interquartile range (IQR), values of  $>1.5$  IQRs but  $<3$  IQRs from the end of the box are labelled as outliers (o), and values more than 3 IQRs from the end of a box are labelled as extreme, denoted with an asterisk (\*).

diabetic group, the cyclosporin unbound concentration values were essentially similar in the two groups ( $0.58 \pm 0.76 \mu\text{g/L}$  in diabetic patients and  $0.52 \pm 0.48 \mu\text{g/L}$  in nondiabetic patients,  $p = 0.59$ ).

#### Blood and Plasma Concentration Ratio

The cyclosporin concentrations in whole blood and in plasma were significantly correlated ( $r = 0.72$ ,  $p < 0.001$ ). Because the B : P ratio varied at each timepoint, the actual values, and not the average value per individual patient, were compared between the two groups. The cyclosporin B : P ratio was  $4.2 \pm 3.9$  for diabetic patients ( $n = 67$  determinations in six subjects) and  $3.4 \pm 1.7$  for nondiabetic patients ( $n = 80$  determinations in seven subjects) [ $p = 0.37$ ]. A positive and significant correlation was observed between the B : P ratio and glycated albumin levels ( $r = 0.37$ ,  $p < 0.001$ ) but not HbA<sub>1c</sub> levels ( $r = 0.03$ ,  $p = 0.66$ ). In addition, B : P ratios were inversely correlated with total protein concentrations, total cholesterol and triglyceride levels.

#### Discussion

Pathophysiological changes associated with diabetes may influence the disposition of pharmacological agents by affecting drug absorption, distribution, metabolism and elimination. These changes may have a profound effect on the disposition of drugs, such as cyclosporin, that exhibit unpredictable absorption, are highly bound to blood and plasma proteins, and are extensively

metabolized by CYP3A enzymes. Until the current investigation, the pharmacokinetics of cyclosporin had never been investigated in diabetic kidney transplant recipients. However, a comparison of cyclosporin  $C_{\text{trough}}$  values in diabetic and nondiabetic kidney transplant patients revealed no significant differences between the two groups.<sup>[23]</sup>

We investigated 12-hour steady-state concentration-time profiles of cyclosporin in whole blood and plasma and measured the cyclosporin  $f_u$ . We also measured the rate of absorption of paracetamol as an indicator of the gastric emptying rate. It was observed that absorption of both paracetamol and cyclosporin was delayed in diabetic patients. The cyclosporin AUC was lower and its  $CL/F_{\text{ss}}$  was numerically higher in diabetic patients. The  $f_u$  of cyclosporin was also higher in diabetic patients; however, the cyclosporin unbound concentration values were comparable. The increased cyclosporin  $f_u$  in diabetic patients suggests that more unbound drug may be available to generate more immunosuppression in diabetic patients. However, lower exposure to the total drug is likely to cancel out the effect of the former. In the absence of a longitudinal clinical study in diabetic and nondiabetic kidney transplant recipients, it will be difficult to draw any clinical conclusion from such pharmacokinetic observations.

Patients with long-term diabetes present with gastric dysmotility or gastroparesis, which consequently delays the gastric emptying rate and slows the rate of absorption of pharmacological agents.<sup>[32]</sup> Paracetamol is a basic compound that is not absorbed in the acidic environment of the stomach, and hence the rate of appearance of paracetamol in the blood would represent the gastric emptying rate.<sup>[33,34]</sup> In our diabetic patients, the paracetamol  $t_{\text{max}}$  was prolonged by approximately 30 minutes, corresponding to an average 35-minute prolongation of the cyclosporin  $t_{\text{max,ss}}$ . We have previously described the pharmacokinetics of mycophenolate mofetil,<sup>[27]</sup> enteric-coated mycophenolate sodium<sup>[35]</sup> and tacrolimus<sup>[36]</sup> in diabetic transplant recipients. Delays were observed in the absorption of cyclosporin, mycophenolate mofetil and tacrolimus, but not enteric-coated mycophenolate sodium.<sup>[35]</sup> The prolongation of the  $t_{\text{max,ss}}$  suggests that the time to achieve maximal immunosuppression may be prolonged in diabetic patients as compared with nondiabetic patients.

Blood and plasma binding of drugs is affected by diabetes because of glycation of serum proteins, including albumin, and increased levels of triglyceride and free fatty acids.<sup>[13,17]</sup> Cyclosporin is a highly lipophilic molecule and is predominantly bound to blood cells in blood (60–65%) and to lipoproteins in plasma (85–90%), leaving a small  $f_u$  of 1–2%.<sup>[37]</sup> In plasma, cyclosporin is predominantly bound to very-low-density lipoprotein [VLDL] (10%), low-density lipoprotein [LDL] (35%) and high-density lipoprotein [HDL] (33%), and a small remaining portion (10–15%) binds to albumin and globulin.<sup>[38]</sup> In our study, there were no significant differences in the concentration of serum

albumin or lipids between diabetic patients and nondiabetic patient. However, we did not measure the concentration of free fatty acids or the haematocrit.

Ciclosporin is a highly protein-bound drug with a low to intermediate hepatic extraction ratio. Therefore, ciclosporin clearance is theoretically dependent upon the  $f_u$  in plasma.<sup>[39]</sup> Due to the reduced binding of drugs in diabetes, the overall hepatic clearance and renal clearance of a highly protein-bound drug may increase,<sup>[13]</sup> leading to increased total drug clearance. We have observed that exposure to ciclosporin, estimated by the  $AUC_{12}$ , was lower and  $CL/F_{ss}$  was higher in diabetic patients. Higher  $f_u$  and lower total concentration values resulted in an almost identical ciclosporin unbound concentration in diabetic patients that is in line with the theory of restrictively cleared drugs.

In a study in streptozotocin-induced diabetic rats, ciclosporin clearance was significantly reduced, indicating that overt diabetes reduces the metabolism of ciclosporin.<sup>[21]</sup> Numerous investigations have reported alterations in the hepatic metabolism of drugs in response to chemical induction of diabetes with alloxan or streptozotocin in laboratory animals.<sup>[40-42]</sup> However, hepatic drug metabolism in diabetic patients has been characterized for only a few compounds.<sup>[19]</sup> Data are absent or minimal on the effects of diabetes on the human CYPs (including CYP3A4, CYP2C9 and CYP2D6) that are of greatest concern in clinical settings,<sup>[19]</sup> and virtually no information is available on the regulation of drug transporters in diabetes. Future studies will be warranted to investigate the concentration of ciclosporin metabolites in diabetic patients and the effect of diabetes and related conditions on the protein expression and messenger RNA levels of drug-metabolizing enzymes and transporters.

A significant drawback of our study was the small sample size in a heterogeneous patient population, prohibiting the detection of statistical significance because of a type II error. Although we tried to match each diabetic patient with a nondiabetic control, based on demographical characteristics, many of the important pharmacokinetic parameters differed numerically but not significantly between the two groups. A future study should either include a larger sample size or investigate ciclosporin pharmacokinetics in diabetic kidney transplant recipients before and after pancreatic transplantation for the treatment of type 1 diabetes.

## Conclusion

The results of this study indicate that diabetic kidney transplant recipients exhibit a higher  $f_u$ , higher  $CL/F_{ss}$ , prolonged  $t_{max,ss}$  and lower AUC values in blood and plasma. A higher  $f_u$  in diabetic patients may translate into an increased immunosuppressive effect of ciclosporin at the lymphocyte level, and a prolongation of the ciclosporin  $t_{max,ss}$  may translate into a delay in maximal immunosuppression.

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Correspondence: Dr *Fatemeh Akhlaghi*, Biomedical and Pharmaceutical Sciences, University of Rhode Island, 125 Fogarty Hall, 41 Lower College Road, Kingston, RI 02881, USA.  
E-mail: [fatemeh@uri.edu](mailto:fatemeh@uri.edu)

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