



Blood Flow Redistribution During Renal Posttransplant Period and its Impact on Cyclosporine Concentration

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SUMMARY. Two independent studies were carried out in order to investigate cyclosporine blood level vs. serum creatinine relationships. Trough drug concentrations (C_0) and concentration-time curves were determined in 8 and 14 renal transplant patients respectively. Apparent drug clearances (CL/F) were calculated following twice daily oral doses. A significant negative correlation between C_0 and serum creatinine was found ($p < 0.01$). Significant positive correlation was also obtained between CL/F and serum creatinine ($p < 0.001$). Mean \pm standard error for serum creatinine and CL/F were: 1.9 ± 0.2 mg.dL⁻¹ and 16 ± 2.1 L.kg⁻¹.day⁻¹ during the first month after transplantation; 1.4 ± 0.2 mg.dL⁻¹ and 11 ± 1.9 L.kg⁻¹.day⁻¹ after 1 month post-graft. After surgery, graft blood supply evolves accordingly the kidney adapts to the recipient subject, which determines the relative blood flow delivered to eliminating organs (liver, intestine). Cyclosporine pharmacokinetics changes throughout the time after the operation and serum creatinine, more than a simple biomarker of renal function, becomes an effective cyclosporine CL/F predictor in kidney recipients.

RESUMEN. "Redistribución del Flujo Sanguíneo luego del Transplante Renal y su Impacto en las Concentraciones de Ciclosporina". Dos estudios independientes fueron llevados a cabo para relacionar creatinina sérica y concentración sanguínea de ciclosporina. En 8 y 14 pacientes transplantados renales respectivamente, se determinaron concentraciones de valle (C_0) y curvas concentración-tiempo luego de administrarles oralmente ciclosporina cada 12 h. Se obtuvo correlación negativa entre C_0 y creatinina sérica ($p < 0,01$). También fue significativa ($p < 0,001$) la correlación positiva encontrada entre la depuración aparente de ciclosporina (CL/F) y la concentración de creatinina. Se obtuvieron los siguientes valores medios \pm error estándar para creatinina sérica y CL/F: $1,9 \pm 0,2$ mg.dL⁻¹ y $16 \pm 2,1$ L.kg⁻¹.día⁻¹ durante el primer mes post-transplante; $1,4 \pm 0,2$ mg.dL⁻¹ y $11 \pm 1,9$ L.kg⁻¹.día⁻¹ luego de 1 mes post-injerto. Desde que el riñón es injertado la irrigación del órgano evoluciona en concordancia con su adaptación al paciente, lo que determina una modificación del flujo sanguíneo relativo dirigido hacia los órganos eliminadores (hígado, intestino). La farmacocinética de ciclosporina cambia así durante el período post-transplante, siendo la creatinina sérica un buen reflejo de la variable depuración de la droga y de la funcionalidad renal del paciente.

INTRODUCTION

Pharmacokinetics of cyclosporine after transplantation

Several articles have been issued regarding the posttransplant day influence on cyclosporine pharmacokinetics. Other covariates are also determinant in the oral clearance values (CL/F, systemic clearance divided by oral bioavailabili-

ty): current body weight and age of patients, their hematocrit, their total bilirubin level, the presence of either inhibitors or inducers of metabolic enzymes; but the time elapsed from the operation is still under debate since its cause remains unclear. Blood levels of drug increase throughout the first three weeks after transplantation due to a decline in the apparent clearance

KEY WORDS: Blood flow redistribution, Cyclosporine; Oral clearance vs. serum creatinine; Renal transplantation.

PALABRAS CLAVE: Ciclosporina; Depuración aparente vs. creatinina sérica; Redistribución de flujo sanguíneo-Transplante renal.

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of drug. Some studies are referred to hematopoietic stem cell transplantation ¹, others to cardiac transplantation ^{2,3}, but most of them were carried out in renal transplant recipients ⁴⁻⁶. But no information is found determining the reasons for such decreasing of the oral clearance.

It seems that cyclosporine inhibits its own systemic and presystemic metabolism that take place both in the liver and in the intestine ⁷⁻⁸. Terminal half-life significantly increased when increasing doses were given to healthy volunteers ⁹. Studies carried out in bone marrow transplant patients, receiving intravenous doses of cyclosporine, revealed that systemic clearance seems to be the main cause for such time-dependent decline in the apparent clearance ¹⁰. Nevertheless, transcriptional down-regulation of P450 cytochrome family enzymes and competitive inhibition of efflux membrane transporters seem to be the reasons for oral bioavailability increase throughout the posttransplant period.

Interaction with sirolimus ¹¹ and grapefruit juice ¹² evidenced that mainly CYP3A4 enzyme and P-glycoprotein transporter (Pgp) ¹³ were both involved in the absorption and pre-systemic elimination of cyclosporine. Substantial increase in the concentration of the cyclosporine metabolites occurred after oral dosing ¹⁰. Even though calcineurin-inhibitor immunosuppressive agent blockades efflux carrier at the membrane ¹⁴, its chronic administration revealed an overexpression of Pgp in the liver ⁷ and other tissues ¹⁵. This overexpression might reduce the impact that the inhibitory effect on Pgp could have on presystemic and systemic clearance. Then, the above mentioned increase of cyclosporine bioavailability could be overestimated. Relative contribution of efflux transporters in drug bioavailability have remained confusing so far ¹⁶⁻¹⁷.

Renal toxicity of cyclosporine and graft rejection

Survival of the engrafted kidney depends on immunosuppressive concentration both at the action site in the lymphocyte and in the kidney, which would be therapeutic and non-toxic respectively. Drug levels below or above the therapeutic range lead to an inappropriate renal blood perfusion ¹⁸⁻²⁰, raising blood levels of substances which are normally eliminated by renal excretion (*i.e.*: creatinine and other endogenous molecules). Arteriolar vasoconstriction in afferent vessels of the kidney seems to be related with intrarenal angiotensin II deposits ²¹, and ei-

ther in low medicated rejection cases or under cyclosporine toxicity an overexpression of Pgp could be involved ^{21,22}.

Differences in efficacy and toxicity among patients could be explained not only by interindividual variability in cyclosporine levels but also in the production of active metabolites. Different attempts were done to investigate metabolic production in patients treated with cyclosporine ^{10,23-24}.

Relative blood flow distribution and drug disposition

Recently ²⁵⁻²⁷, a new perspective in mass transfer considering cardiovascular physiology influence on first order rate constant of drugs was developed. According to this way of forecasting drug motion through blood / tissue barriers, if renal blood flow fraction decreases, the overall extra-renal flow fraction increases, and then an enhanced drug diffusion outside the vessels will take place in different organs. In the case of cyclosporine, renal toxicity and/or kidney rejection would lead to a higher clearance of drug because of the increased blood flow fraction delivered to the liver and/or at the intestine. This has already been reported by our group in renal transplant patients followed up for a long period of time ²⁸⁻²⁹, measuring trough drug levels.

Cyclosporine oral clearance was related to serum creatinine in clinical stable patients engrafted for at least 3 months ³⁰. However, this relationship was not believed to be supported physiologically and then it was discarded. In a multivariate analysis creatinine clearance had significant positive correlation with the area under the curve of blood levels of cyclosporine ³¹. Nephrotic and kidney-transplanted children displayed a significant negative correlation between creatinine clearance and cyclosporine clearance ³². All these reports are in agreement with the blood flow redistribution theory, the higher liver blood flow fraction becomes, as a consequence of its reduction in the kidney, the more extensive cyclosporine is cleared from the body. Hepatic blood supply is determinant in cyclosporine clearance as it was reported in a liver-transplanted child when he was treated from hepatic venous stenosis ³³.

Even though last paragraph retrieves important evidences from the literature, we will describe in this communication the experimental relationship found between serum creatinine and cyclosporine oral clearance or cyclosporine

blood level, in renal transplant patients followed up during the first year after transplantation.

PATIENTS AND METHODS

Patients and doses

Two independent trials were carried out. In a first preliminary study (trial-1), 8 renal transplant patients were followed up during 1 month after the operation at the Clinical and Provincial Hospital in Barcelona (Spain). Morning trough (C_0) blood levels were weekly monitored in this critical post-graft period as routine practice in order to associate them with the clinical and biochemical outcomes of graft recipients. Laboratory parameters related with the liver and kidney functions of patients, were determined but for the purpose of this communication only serum creatinine was retrieved. Oral treatment with cyclosporine microemulsion (Sandimmun-Neoral, Novartis) was started after surgery with doses of $10 \text{ mg.kg}^{-1}.\text{day}^{-1}$ approximately.

In a second two-year prospective study (trial-2), adult renal transplant patients, treated with cyclosporine - mycophenolate mofetil - steroids, were monitored during the first year post-graft according to the University Hospital protocol in Montevideo (Uruguay). Two-hour cyclosporine blood concentration (C_2) was used as guideline for dose adjustment^{34,35}. Patients were given orally $8 \text{ mg.kg}^{-1}.\text{day}^{-1}$ approximately, twice daily, during the first week, and thereafter the individual dose was adjusted conveniently. Original (Sandimmun-Neoral [Novartis]) and average bioequivalent brands of cyclosporine microemulsion (Sigmasporin-Microoral [Novaquímica Nature's Plus Farmaceutica]) were used according to the supplier availability (National Resource Center). Before switching a patient from the formulation he was receiving, a concentration-time curve (AUC) was determined. Two days afterwards, one-hour (C_1) and two-hour cyclosporine blood levels were obtained in order to check any deviation from the established steady-state. Fourteen cadaveric kidney recipients (aged 23-65 years) were recruited, after receiving their informed consent, and serial blood samples from 0 to 4 hour post-dose were withdrawn.

Follow-up of cyclosporine blood concentration and data processing

In trial-1, trough blood levels were determined by enzyme-multiplied immunoassay technique (EMIT, Dade-Behring Diagnostics, Milton Keynes, UK). In trial-2, cyclosporine blood level

- time curves were determined provided the same dose was kept for at least 5 days. Cyclosporine levels were measured using fluorescence polarized immunoassay (FPIA, TDx Abbott Laboratories, Illinois, USA).

In order to calculate the apparent clearance (CL/F) of cyclosporine, an estimation of the area under the curve from zero to twelve hours (AUC₀₋₁₂) was done considering blood level at the end of the administration interval to be similar to the pre-dose value. AUC was calculated using the trapezoidal rule. Then, CL/F was assessed as the dose administered every 12 h divided by AUC₀₋₁₂. In both trials, serum creatinine levels of patients, corresponding to the same day of cyclosporine determinations, were obtained from their clinical history.

Data coming from trial-2 were divided into two groups: parameters obtained during the first month after surgery (group A) and parameters obtained between one month and one year after transplantation (group B). Mean and standard error (SE) were calculated. Non-paired Student t-test was used for mean comparisons provided each series of data showed normal distribution and similarity of variances.

Linear regressions were determined between C_0 (trial-1) or CL/F (trial-2) and serum creatinine. Significance for differences between means and regression lines will be assessed when type-I error has a probability below five percent ($p < 0.05$), otherwise non-significant (NS) will be informed.

RESULTS

Figure 1 shows mean (\pm SE) cyclosporine blood level curves obtained in trial-2 during the first month (group A, $n=7$) and after (group B, $n=7$) one month posttransplantation. Even though blood levels at the end of the interval (evening hours) might be lower than pre-dose ones (morning hours)³⁶, AUC₀₋₁₂ yielded comparable values to the previously reported³⁷, and then CL/F could be consistently assessed: 16 ± 2.1 and $11 \pm 1.9 \text{ L.kg}^{-1}.\text{day}^{-1}$ (NS), for groups A and B respectively. Oral clearance decreased after one month post-graft in the same way serum creatinine did (1.9 ± 0.2 and $1.4 \pm 0.2 \text{ mg.dL}^{-1}$ [NS], groups A and B respectively).

A significant positive linear regression (Fig. 2) was found between CL/F and serum creatinine ($p < 0.001$). It should be noted that even though higher serum creatinine values are frequently observed during the first month after transplantation, when patients are not stabilized

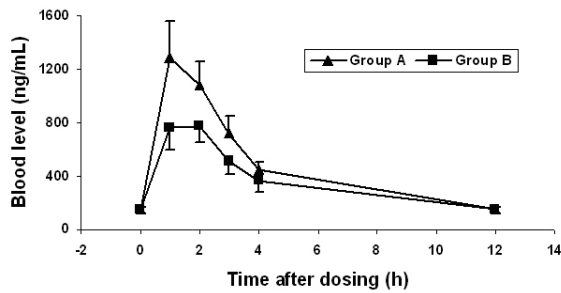


Figure 1. Mean (\pm standard error) cyclosporine blood concentration curve obtained during the interval of administration (12 hours), in the first month after kidney transplantation (mean dose of $7.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$: group A, $n=7$) and between 1 month and 1 year post-graft (mean dose of $3.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$: group B, $n=7$). 12-hour post-dose levels were assumed to be the same as pre-dose ones.

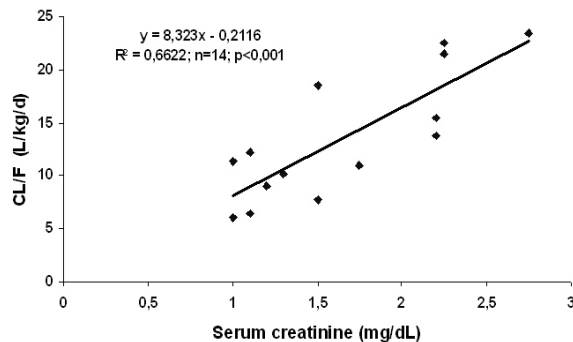


Figure 2. Cyclosporine apparent clearance (CL/F) versus serum creatinine relationship, obtained in fourteen renal transplant patients after oral administration during one-year post-graft period.

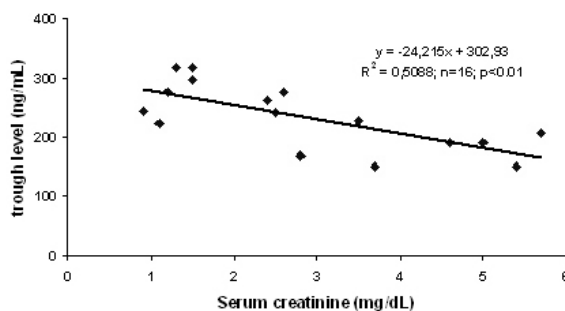


Figure 3. Cyclosporine trough blood concentration versus serum creatinine relationship, obtained in eight renal transplant patients during the first month after surgery.

yet, some high values are also found after several months of treatment.

Higher serum creatinine values by the first month after surgery were also well documented in trial-1 ($2.9 \pm 0.4 \text{ mg}\cdot\text{dL}^{-1}$). Figure 3 shows the negative linear regression ($p<0.01$) found in this trial between C_0 and serum creatinine. Graph in

this figure was constructed using two C_0 points for each patient provided the same dose was kept, and trough blood levels were dose-normalized assuming all patients were receiving the same average daily dose. In all cases the same patient displayed higher cyclosporine level when serum creatinine decreased and viceversa. In other words, some patients showed increased serum creatinine a long time posttransplantation (sign of clinical worsening due to renal drug toxicity or graft rejection).

Trial-2 evidenced non bioequivalent C_1 and C_2 values between formulations in several patients, even though both brands were assessed as average bioequivalent³⁸. This is not surprising because average bioequivalence does not assure switchability in all individuals, and due to the narrow therapeutic range of cyclosporine, interchangeability between average bioequivalent formulations has been discouraged³⁹. Tendency in this trial was on the way to inform a lower and/or slower absorption profile when Sigmasporin-Microoral was administered. C_1 and/or C_2 were far from a $\pm 20\%$ interval around the respective value obtained in the concentration-time curve. Most of these patients moved the time to reach maximum concentration from 1 to 2 h when switched from Sandimmun-Neoral to Sigmasporin-Microoral, and viceversa.

DISCUSSION AND CONCLUSIONS

Trial-1 showed a higher, but non significant, mean serum creatinine value than that observed in trial-2 group A. This higher value is because cyclosporine blood concentrations shortly after the operation were analyzed in trial-1.

Although dose-normalized C_2 values obtained in trial-2 for the same patients are not reported here, a high intraindividual variability similar to the AUC-dose-normalized interindividual variability was observed. This is in agreement with the very important changes that patient-cyclosporine pharmacokinetic system had during the first year after transplantation. Hence, difference among individuals at the same post-transplant time could be as important as inpatient differences throughout time.

According to our results, formulation exchange in the same patient during cyclosporine treatment should be avoided. Data on file showed us that Sigmasporin-Microoral is prone to deliver the drug slower than Sandimmun-Neoral but maintaining the extent of absorption apparently. However, this tendency should be tested more rigorously using appropriate experi-

mental designs with stabilized transplant patients. Bearing all this in mind and being concerned that patients were off from interacting co-medications at the moment AUCs were determined, CL/Fs calculated in trial-2 could be reliable enough to be considered in analysis of correlation as it was done in the present communication.

Significant correlations obtained between cyclosporine CL/F or cyclosporine C_0 and serum creatinine are consistent one to each other. In other words, it could be assessed that cyclosporine clearance, which is mainly eliminated from the splanchnic region, and creatinine clearance, which is eliminated by the kidneys, run inversely. The higher creatinine clearance is, the lower cyclosporine clearance becomes, or the higher renal function is, the lower intestinal and hepatic ability to clear cyclosporine from the body becomes. So, our hypothesis based on blood flow redistribution among the organs and its impact on drug pharmacokinetics could be satisfactory demonstrated by these two experiments performed with this high hepatic extrac-

tion drug⁴⁰. However, blood flow redistribution, as a consequence of renal function normalization, becomes inadequate to explain posttransplant CL/F decrease when other organs apart from the kidneys are engrafted^{1-3,10,41}.

Maybe both metabolic inhibition and hemodynamic adaptation share responsibility for cyclosporine CL/F decline throughout the first month after surgery in renal transplant patients. Nevertheless, cardiac output redistribution still remains as a reliable mechanism for CL changes due to drug toxicity or graft rejection even beyond that period of time. Then, more than a clinical outcome, serum creatinine becomes also a useful biomarker of cyclosporine systemic clearance.

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