Trimetazidine does not modify blood levels and immunosuppressant effects of cyclosporine A in renal allograft recipients

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Aims In renal allograft recipients, trimetazidine (Vastarel®) was proposed to be associated with the classic immunosuppressant treatments because it displays anti-ischaemic effects which may protect against cyclosporine A nephrotoxicity. The objective of this work was to assess the possibility of coadministering cyclosporin A, Sandimmun®, and trimetazidine.

Methods Twelve renal transplant patients were selected on the basis of the stability of their cyclosporine A blood concentrations for the previous 3 months. They received trimetazidine, 40 mg twice daily orally for 5 days. Other coadministered drugs were kept unchanged during the study. Before and after trimetazidine administration, cyclosporine A blood concentrations, plasma interleukin-2 and soluble interleukin-2 receptor levels were measured.

Results The data showed that neither cyclosporin A blood pharmacokinetic parameters, C_{max}, t_{max}, AUC, nor the concentrations of interleukin-2 and soluble interleukin-2 receptors were significantly modified.

Conclusions Therefore, it was suggested that trimetazidine may be coadministered with cyclosporine A without cyclosporine A dosage adjustment.

Keywords: cyclosporine A, trimetazidine, interleukin-2, soluble interleukin-2 receptors

Introduction

Trimetazidine (TMZ), 1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride, is an anti-ischaemic drug mainly used in coronary heart disease [1, 2]. Recently, Creagh *et al.* [3] have shown that TMZ is also able to inhibit the acute nephrotoxic effects of cyclosporine A (CsA) in a canine kidney model. This protective effect of TMZ was attributed to its anti-ischaemic properties. Furthermore, Salducci *et al.* [4] have demonstrated that TMZ could restore ATP synthesis of isolated rat liver mitochondria previously impaired by a Ca^{2+} overload either alone or associated to CsA. These data suggest that TMZ may have a beneficial effect on nephrotoxicity induced by CsA.

Our interest in such TMZ properties was recently strengthened by the observation that TMZ did not alter immunosuppressant effect of CsA as shown *ex vivo* by lymphoproliferative assays on human lymphocytes and *in vivo* by the delayed hypersensitivity model in mice [5].

However these data needed to be confirmed *in vivo* in humans. Moreover, the possibility of a pharmacokinetic interaction between the two drugs altering CsA blood levels could not be excluded *a priori*. Indeed, CsA is thoroughly biotransformed in liver by isoenzymes of the cytochrome P450 (CYP) 3A subfamily [6, 7]. TMZ is also biotransformed in the body but to a lesser extent than CsA [8]. As at least 10 TMZ metabolites have been identified in human urine [8], involvement of CYP3A isoenzymes in TMZ biotransformations was a likely hypothesis. All these data prompted us to run a pilot study in patients to check the outcome of the pharmacokinetics and immunosuppressant effect of CsA when TMZ is given in combination. This work focused on CsA alone, because TMZ has a high therapeutic ratio which prevents the patients from any risk of overdosage. We used renal allograft recipients whose drug dosage regimens were stabilized on the basis of CsA trough concentration measurements. In this pilot study, TMZ was added over a short period of time, although long enough to reach steady-state plasma levels ($t_{1/2} = 6.60$ h) [9]. CsA blood levels and concentrations of markers of its immunosuppressant effect, interleukin-2 (IL-2) and soluble interleukin-2 receptors (RIL-2S), were simultaneously measured [10].

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Methods

Clinical

Twelve renal transplant patients (seven men and five women, mean age 42 years, range 19–66), 8 to 53 months post-kidney transplantation, participated in this study. They were asked to maintain their usual eating, drinking and smoking habits throughout the study. All patients had a three-drug course of immunosuppressive therapy consisting of CsA,

prednisone (0.20 ± 0.07 mg kg^{-1} day^{-1}) and azathioprine (0.90 ± 0.46 mg kg^{-1} day^{-1}), except one patient who had no azathioprine. Throughout the study the drugs and doses of immunosuppressive therapy remained unchanged. The protocol was approved by the 'Comité consultatif de Protection des Personnes dans la Recherche Biomédicale de Marseille 1—ethics committee' (10 June 1993) and a written consent was obtained from each patient.

The study was divided into two periods A and B and carried out over 8 consecutive days for every patient who was confined in an investigational unit at days 4 and 8 of the study. CsA was administered orally twice daily at 08.00 h and 20.00 h throughout the study, i.e. period A and B. The dose of CsA differed between the patients (mean: 5.9 mg kg^{-1} day^{-1}—range: 3.2–9.3 mg kg^{-1}) but remained the same for each during the study. During period A (days 1–3 and day 4 until 08.00 h), patients did not receive TMZ. During period B, from day 4 at 20.00 h to day 8 at 20.00 h, oral doses of TMZ were given in combination with CsA. Patients received 20 mg TMZ every 12 h at 08.00 h and 20.00 h each day.

Blood samples (5 ml) were collected in tubes containing ethylene diamine tetraacetic acid from day 1 to day 9 before the morning dosing for the determination of plasma RIL-2S, plasma IL-2 and blood CsA concentrations. At day 4 (CsA treatment) and at day 8 (CsA + TMZ), blood samples were withdrawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h after the morning dosing for the determination of blood CsA concentrations. Aliquots of plasma were obtained after centrifugation at 13 000 *g* for 15 min. Blood and plasma samples were stored at -20° C until analysis. Blood CsA concentrations were measured by specific monoclonal radioimmunoassay (monoclonal Cyclo-trac SP ^{125}I-RIA kit, Incstar corporation). The intra-day coefficients of variation were less than 11% for three concentrations: 46 μg l^{-1}, 186 μg l^{-1} and 625 μg l^{-1}. The inter-day coefficients of variation were between 4.6 and 10.0% for three concentrations from 71 to 505 μg l^{-1}. The limit of detection was 6 μg l^{-1}. This method only measures CsA and demonstrates no significant cross-reactivity with its metabolites (below 1.7%). Plasma IL-2 concentrations were determined by immunoradiometric assay (Medgenex Diagnostics SA) and RIL-2S were assayed by using an immunoenzymatic technique (Immunotech International SA).

Creatinine clearance was estimated from plasma creatinine concentration (measured at day 1 and 10) by use of the Cockcroft & Gault equation [11].

Data analysis and statistics

The plasma concentration-time profiles of CsA were analyzed for area under the concentration-time curve (AUC(0, 12 h)) from 0 to 12 h post-dosing, observed peak plasma concentration (C_{max}), and observed time to reach C_{max} (t_{max}). The AUC(0, 12 h) was calculated by the trapezoidal method.

Statistical comparisons between the pharmacokinetic parameters of CsA at day 4 (period A) and day 8 (period B) were done by using the Friedman test for t_{max} [12], and ANOVA for C_{max} and AUC(0, 12 h). Similarly, comparisons between IL-2, RIL-2S, trough (pre-dose) CsA concentrations and calculated creatinine clearance were by ANOVA. 95% confidence intervals of the difference in means of all these parameters were also calculated. Coefficients of variation for inter- and intraindividual variability were computed using the analysis of variance tables, including between-subjects and between-periods sources of variation, obtained successively with and without TMZ therapy.

Results

The steady–state blood CsA concentration *vs* time profiles over a 12 h dosing interval during period A (control period without TMZ) and period B (with TMZ) are shown in Figure 1. Mean values for t_{max}, C_{max}, and AUC(0, 12 h) referable to the morning CsA dose on day 4 (period A) control period and on day 8 (period B) are listed in Table 1.

Mean morning trough CsA levels (C_{ssmin}) from day 1 to day 4 and from day 5 to day 8 are also reported (Table 1). Statistical analysis did not show any significant differences between the two periods for any parameter. Coefficients of variation for inter- and intraindividual variability are the following: 62.88%, 39.37% without TMZ and 56.81%, 29.67% with TMZ respectively.

Similarly, plasma IL-2 and RIL-2S concentrations did not show any significant changes between period A and B (Table 1).

In addition, creatinine clearance measured at day 1 and 10 was not significantly modified (D1: 42.0 ± 11.5 ml min^{-1}; D10: 41.2 ± 10.6 ml min^{-1}; $P > 0.05$).

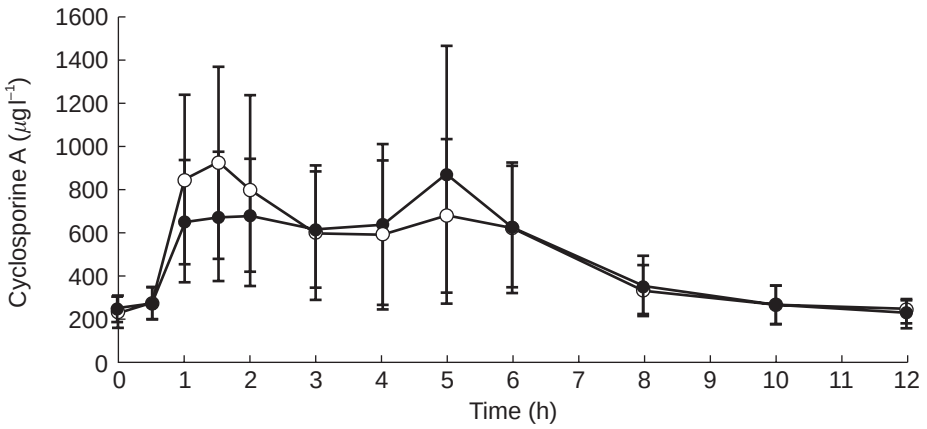

Figure 1 Mean steady-state blood cyclosporine A concentrations (mean ± s.d.). The profile (○) is obtained at day 4 with cyclosporine A alone, while the other (●) is obtained at day 8 with the coadministration of cyclosporine A and trimetazidine.

Table 1 Mean (s.d.) steady-state cyclosporine A parameters without and with trimetazidine.

	Cyclosporine A parameters			
	Without trimetazidine	*With trimetazidine*	P *values*	*95% CI**
t_{max} (h)	2.9 (2.0)	3.3 (1.7)	NS	−45.3 to 74.7%
C_{max} ($\mu g\,l^{-1}$)	1188 (305)	1133 (438)	NS	−29.8 to 20.5%
AUC(0, 12h) ($\mu g\,l^{-1}\,h^{-1}$)	5829 (1832)	5846 (1731)	NS	−17.7 to 18.3%
$C_{ss\,min}$ ($\mu g\,l^{-1}$)	232 (84)	251 (91)	NS	−2.2 to 18.4%
IL-2 (iu ml^{-1})	0.33 (0.21)	0.33 (0.19)	NS	−17.7 to 14.3%
RIL-2S (pmol l^{-1})	87.4 (62.2)	91.5 (61.8)	NS	−2.1 to 11.6%

*95% CI: 95% confidence intervals for the difference of means. IL-2: interleukin 2; RIL-2S: soluble interleukin-2 receptor.

Discussion

These data show that neither CsA blood concentrations, nor plasma levels of markers of its immunosuppressant effect such as IL-2 and RIL-2S, are significantly modified by TMZ 80 mg day^{-1}. From a pharmacokinetic point of view, these results suggest that TMZ biotransformations should not be CYP 3A4 dependent and thus should not interfere with the metabolic pathways of CsA at the selected dosages of each drug. The CsA concentrations measured in this study were subject to a wide intra- and interindividual variability. However, such variability is similar to that previously reported for CsA itself [13, 14].

As no sign of transplant rejection was observed during the trial, the evaluation of CsA immunosuppressant effect was based on the measurements of T-cells functional markers [15–17]. In our patients, IL-2 and RIL-2S concentrations were similar to those of a population without any lymphocyte activation (0.4 u ml^{-1} and 0.8 pM for IL-2 and RIL-2S respectively). Inhibition of lymphocyte activation by CsA remained unchanged under TMZ + CsA treatment as shown by the stability of IL-2 and RIL-2S concentrations. As the inhibition of IL-2 production by CsA occurs 4 to 12 h after the C_{max}, the two periods of the survey were long enough to see a variation in these parameters [10]. When we considered each patient alone, no progressive rise (> 20% patient's baseline) of IL-2 and RIL-2S in two or more consecutive samples were found. Confidence intervals of the difference in means of IL-2 and RIL-2S concentrations, between the two phases of the study confirm the absence of any modification (Table 1). This conclusion is in accordance with previous data showing the lack of modification of CsA ability to inhibit T-cell activation, when it is associated to TMZ [5].

In summary, our data indicate that under our experimental conditions TMZ had no significant effect either on the pharmacokinetic parameters of CsA or on IL-2 and RIL-2S concentrations. Therefore, our results suggest that both drugs may be coadministered and that there is no need for additional CsA dosage adjustment when they are given in combination.

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