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Whole blood cyclosporin monitoring in liver and heart transplant patients: evaluation of the specificity of a fluorescence polarization immunoassay and an enzyme-multiplied immunoassay technique

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Abstract

The specificity of two cyclosporin immunoassays were evaluated. Eleven patients were followed for the first four weeks after heart ($n = 3$) or liver ($n = 8$) transplantation. Cyclosporin A (CsA) monitoring was performed concomitantly by a monoclonal fluorescence polarization immunoassay (mFPIA) and enzyme-multiplied immunoassay technique (EMIT®) during this period. For several patients, cyclosporin monitoring was also performed by high performance liquid chromatography (HPLC) or by polyclonal fluorescence polarization immunoassay (pFPIA). Liver function was assessed by follow-up of plasma total bilirubin, γ-glutamyl transferase and alkaline phosphatase and renal function by plasma creatinine. All the patients presented episodes of impaired liver function. Higher CsA levels were found using mFPIA measurements as compared to the EMIT® measurements (ratio mFPIA:EMIT® (medium range) = 1.4 (1.0–2.3)). A higher degree of cross-reactivity of the antibody used in the mFPIA as compared to the EMIT® was demonstrated by specific measurements of CsA and its primary metabolite, AM1, by HPLC. © 1997 Published by Elsevier Science B.V.

Keywords: Immunoassay; HPLC; Transplantation

1. Introduction

To minimize nephrotoxicity and optimize immunosuppression, regular monitoring of whole blood Cyclosporin A (CsA) concentration has been recommended [1]. Recent consensus documents concluded that the measurements of CsA should be made in whole blood using a method with a high specificity for the parent compound [2]. The immunosuppressive activity of the characterized metabolites is much less than the parent compound [3,4] and until now it has not even been possible to ascertain whether high metabolite

Abbreviations: CsA, cyclosporin A; mFPIA, monoclonal fluorescence polarization; EMIT, enzyme-multiplied immunoassay technique; pFPIA, polyclonal fluorescence polarization.

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concentrations or particular metabolites were the cause of renal dysfunction [5]. Some comedicated drugs act on the cytochrome P450 isoenzymes, like CsA does, and so influence the metabolic pattern of CsA resulting in a lower CsA concentration and a higher metabolite concentration in blood [6,7]. In this context, measurement of cyclosporin with a highly specific method is therefore essential [1].

High performance liquid chromatography (HPLC) is a very selective technique, but rather time-consuming, by which CsA and its metabolites can be quantified [8]. Fluorescence polarisation immunoassay (mFPIA; Abbott Diagnostics) [9], enzyme multiplied immunoassay technique (EMIT®; Syva) [10], radioimmunoassay (RIA; INCSTAR) [11], all techniques using monoclonal antibodies, have almost replaced HPLC. The real specificity of these immunoassays has been questioned: variable cross-reactivities of the CsA-antibody with metabolites have been demonstrated for each of these techniques and might necessitate adaptation of the therapeutic range for blood cyclosporin concentrations [10,12].

The purpose of this study was to evaluate the amplitude of the overestimate of parent cyclosporin concentrations measured in whole blood by two immunoassays, i.e. mFPIA and EMIT®, and the impact of this overestimate on the therapeutic range of CsA blood levels in situations of impaired liver function, i.e. heart and liver transplant recipients.

2. Experimental

2.1. Patients

Three heart and eight liver transplant patients were monitored for a period of 4 weeks post-transplantation. Patients received a triple immunosuppressive therapy with prednisone, azathioprine, and CsA. CsA dosage was adjusted to achieve target levels of: heart transplant patients, 250–350 μg l^{-1}; first four liver transplant patients, 100–200 μg l^{-1}; last four liver transplant patients, 250–350 μg l^{-1} for the two first weeks and 200–300 μg l^{-1} for weeks three and four.

2.2. Specimen collection

EDTA-anticoagulated whole-blood specimens were obtained just before the morning daily dose of CsA. The blood was collected from a frozen vein and was stored refrigerated until analysis within one week.

2.3. CsA assays

The routine mFPIA method (Abbott, Abbott Park, IL) was compared to the EMIT® method (Syva, Brussels, Belgium). These techniques were performed according to the manufacturer's instructions with a minor modification to the mFPIA technique: the calibrators range from 50 to 1000 μg l^{-1} in place of 100 to 1500 μg l^{-1} (calibrator 100 μg l^{-1} has been diluted two times with calibrator 0 μg l^{-1}). In one heart and one liver transplant patient, a non-specific cyclosporin assay (pFPIA; Abbott), using non specific polyclonal antibodies, was also performed. HPLC analysis of CsA and some metabolites were performed by a modification of the method reported by Carruthers et al. [13]. Sample preparation modifications included the substitution of CsC as the internal standard and a heptane wash after reconstitution of the extract in 300 μl ammonium-sulfate (2% w/v). The chromatographic separation was performed in the reversed-phase mode using an ultrasphere octyl 5 μ column (25 cm × 4 mm i.d.) (Beckman, San Ramon, CA) at 75°C with acetonitrile 270:water 80:methanol 380:2-propanol 5 as the mobile phase at 2 ml min^{-1}. UV detection was set at 210 nm. The peak height ratio method was used for quantification. The CsA metabolites were provided by Sandoz (Basel, Switzerland).

2.4. Control samples

Commercially available whole-blood CsA control Level-1 and Level-2 (Biorad Laboratories, Anaheim, CA) were used to assess the quality of CsA analysis.

Table 1
Longitudinal study in heart (Htx) and liver (Oltx) transplant patients

Patients	mFPIA-CsA (μg l^{-1})	EMIT-CsA (μg l^{-1})	Ratio mFPIA:EMIT	Creatinine (<106) (μmol l^{-1})	Total bilirubin (<20) (μmol l^{-1})	ALP (<250) (U l^{-1})	GGT (<40) (U l^{-1})
Htx 1 (n = 15)	250 (195–460)	174 (130–356)	1.4 (1.1–1.6)	133 (106–283)	28 (27–38)	201 (188–292)	N.D.
Htx 2 (n = 20)	315 (149–480)	203 (100–326)	1.5 (1.3–1.8)	256 (186–442)	24 (15–39)	213 (134–386)	128 (67–148)
Htx 3 (n = 25)	320 (130–960)	243 (86–678)	1.5 (1.2–1.6)	124 (97–177)	19 (10–41)	212 (157–336)	189 (125–265)
Oltx 1 (n = 28)	115 (31–165)	71 (21–149)	1.3 (1.1–1.8)	194 (71–380)	34 (17–99)	253 (24–426)	212 (130–478)
Oltx 2 (n = 23)	150 (46–242)	105 (37–172)	1.4 (1.0–2.3)	133 (88–203)	28 (19–150)	119 (84–154)	48 (24–92)
Oltx 3 (n = 17)	190 (18–275)	124 (11–160)	1.4 (1.0–1.8)	133 (53–177)	150 (38–684)	305 (130–509)	165 (34–251)
Oltx 4 (n = 25)	165 (69–258)	109 (57–160)	1.3 (1.1–1.8)	88 (71–106)	14 (10–75)	152 (115–258)	41 (33–261)
Oltx 5 (n = 26)	190 (76–535)	126 (62–412)	1.4 (1.1–1.8)	186 (150–309)	21 (7–97)	243 (155–554)	96 (58–227)
Oltx 6 (n = 28)	260 (105–495)	212 (49–403)	1.5 (1.1–2.2)	124 (80–141)	256 (111–403)	410 (138–1010)	410 (84–915)
Oltx 7 (n = 28)	260 (60–455)	170 (24–309)	1.4 (1.1–1.8)	71 (44–124)	201 (133–554)	322 (154–579)	259 (107–417)
Oltx 8 (n = 19)	250 (125–650)	201 (75–492)	1.3 (1.2–1.7)	88 (71–159)	22 (12–39)	75 (55–169)	34 (19–126)

CsA blood levels measured by mFPIA and EMIT®, the ratio mFPIA-CsA:EMIT®-CsA, plasma creatine, total bilirubin, alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) levels. Values are expressed as median and (range).

2.4.1. Enzymes, total bilirubin, and creatinine assays

Alkaline phosphatase (EC 3.1.3.1) and γ-glutamyl transferase (EC 2.3.2.2) were measured in plasma at 30°C by IFCC-recommended methods, with commercially available kits (Boehringer Mannheim, Mannheim, Germany). Plasma total bilirubin and creatinine levels were measured by a Jendrassik and a kinetic Jaffé procedure, respectively.

2.5. Statistical analysis

For the statistical evaluation of method comparison a parametric rank procedure, i.e. Passing-Bablok [14], and a simple linear regression were used.

3. Results

3.1. Precision of the techniques

Interassay coefficient of variation for the mFPIA method (n = 36) was 4.3% at level 1 (mean = 99 μg l^{-1}) and 4.8% at level 2 (mean = 252 μg l^{-1}), and for the EMIT method (n = 18) was 8.5% at level 1 (mean = 93 μg l^{-1}) and 7.7% at level 2 (mean = 218).

3.2. Longitudinal study

CsA whole-blood trough concentration was measured in three heart and eight liver transplant patients during the first three to four weeks after transplantation. Significantly higher concentrations of whole-blood CsA were obtained by the specific mFPIA as compared to the specific EMIT® (median = 1.4; range 1.0–2.3) (Table 1).

In one heart and three liver transplant patients (n = 17), the higher degree of cross-reactivity (with CsA metabolites) of the monoclonal antibody used in mFPIA was demonstrated by the comparison of whole-blood CsA measurements performed by EMIT or mFPIA against those performed by HPLC. Typical results of the comparisons of CsA specific immunoassays measurements, in one heart and one liver transplant patient, with the nonspecific pFPIA assay and

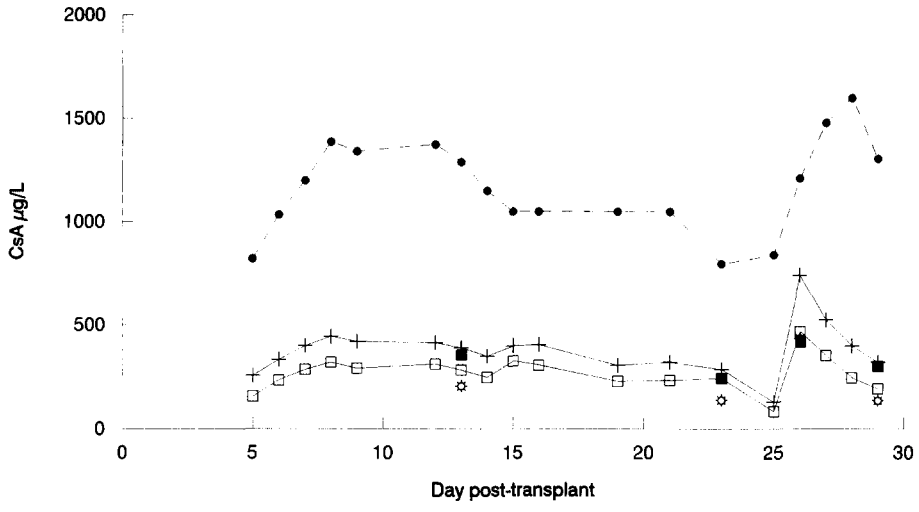

Fig. 1. Follow-up of CsA whole blood trough concentrations determined by different methods in a heart transplant patient. (□, EMIT; +, mFPIA; ●, pFPIA; ☆, HPLC-CsA; ■, AM1)

HPLC CsA and metabolite measurement are represented in Figs. 1 and 2, respectively. As represented in Fig. 3, EMIT®-CsA measurements compared to HPLC-CsA measurements showed no deviation of slope from 1.0 (1.090; interval 0.784–1.604) and no deviation of the intercept from 0.0 (16.416; interval −26–34); the mFPIA-CsA measurements compared to HPLC-CsA measurements showed a significant deviation of slope from 1.0 (1.76; interval 1.41–2.39) and no deviation of the intercept from 0.0 (18.3; interval −42.8–48.5). A significant cross-reactivity of the monoclonal antibody used in mFPIA with AM1 is indicated in Fig. 4 (paired t-test: 3.88; $n = 13$; $P \leq 0.01$).

3.3. Liver function

Each patient presented episodes of impaired liver function as assessed by plasma total bilirubine, alkaline phosphatase or γ-glutamyl transferase levels higher than the upper reference limit (Table 1).

3.4. Renal function

Among the eleven transplant patients followed, after a return of creatinine concentrations to the normal reference range, one heart and four liver transplant patients presented episodes of nephrotoxicity ($n = 8$) as assessed by creatinine levels above the upper reference limit of 106 µmol l^{-1} between day 2 and day 28. Correlation between EMIT®-CsA and mFPIA-CsA showed a slope of 1.33 (interval 1.26–1.40) at creatinine levels < 106 µmol l^{-1} and a slope of 1.39 (interval 1.32–1.47) at creatinine levels > 106 µmol l^{-1} (Fig. 5).

4. Discussion

The data from our longitudinal study show differences in estimation of CsA whole blood levels by mFPIA or by EMIT® in the immediate post-operative period of heart and liver transplant patients. The results achieved with mFPIA were significantly higher than with EMIT for both groups of patients. We have demonstrated here a higher degree of cross-reactivity, especially with metabolite AM1, of the monoclonal antibody used in mFPIA compared to the antibody used in EMIT® (Figs. 1–4).

All the patients studied presented episodes of impaired liver function, a clinical situation that might, itself, lead to accumulation of CsA metabolites. We found an overestimate of 40% (range 0–220%), by mFPIA relative to EMIT® in the samples tested. Large intra- and interindividual variabilities in CsA results were also demonstrated.

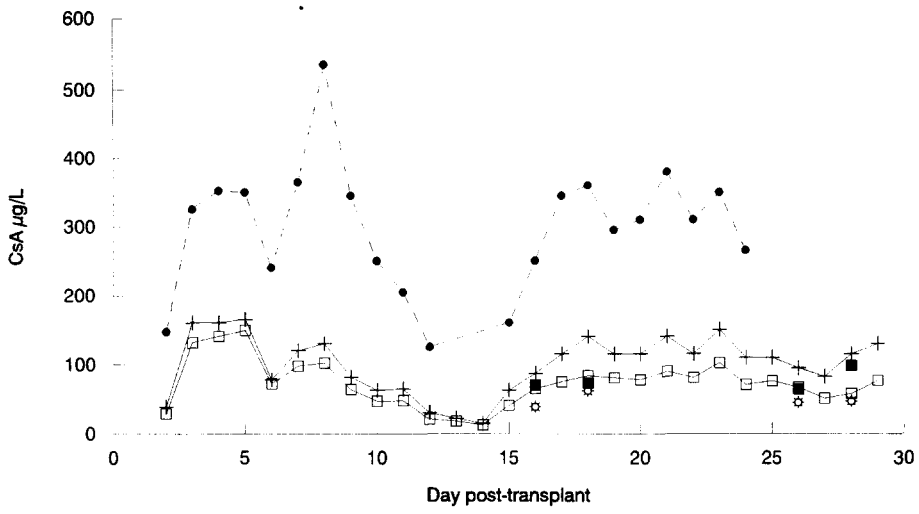

Fig. 2. Follow-up of CsA whole blood trough concentrations determined by different methods in a liver transplant patient. (□, EMIT; +, mFPIA; ●, pFPIA; ☆, HPLC-CsA; ■, AM1)

Some authors have reported large variabilities in the level of cross-reactivity of the antibody anti-cyclosporin used in immunoassay techniques with CsA metabolites [10,12]. Depending on the transplant population studied, i.e. paediatric, liver or heart, other authors have also reported high variability in the level of CsA overestimate [15,16]. One explanation of all these findings might be variability in the level of impaired liver function, transplantation type and time following transplantation. All of these factors could influence the concentration of different cyclosporin metabolites in the blood. Especially for patients with impaired

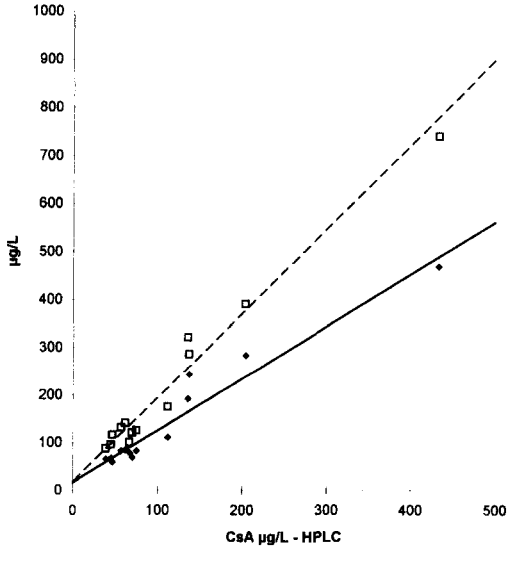

Fig. 3. Comparative analysis of CsA whole blood levels obtained by specific EMIT® (◆), mFPIA (□) and HPLC.

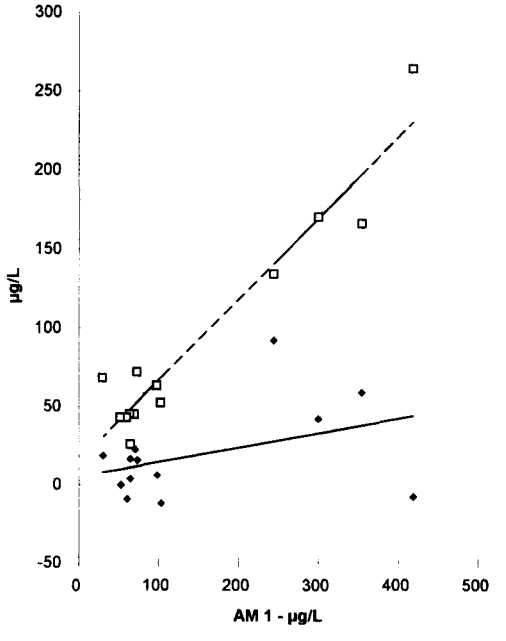

Fig. 4. Correlation between whole blood concentrations of AM1 measured by HPLC and (◆) EMIT-CsA levels minus HPLC-CsA levels or (□) mFPIA-CsA minus HPLC CsA.

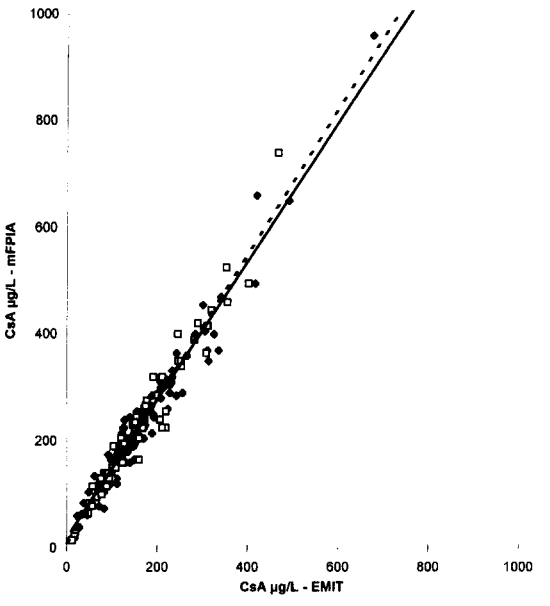

Fig. 5. Correlation between EMIT®-CsA and mFPIA-CsA for creatinine < 106 µmol (◆) and creatinine > 106 µmol l^{-1} (□).

CsA metabolite excretion, disproportionately high and variable amounts of cross-reacting metabolites might account for an increased positive and variable bias in CsA determination particularly when measured by mFPIA. In such cases, it seems unreasonable to simply adjust the therapeutic range of 'cyclosporin' as measured by mFPIA. There might be a risk of underimmunosuppression. Another measurement method with a high(er) specificity for cyclosporin parent compound, like EMIT®, might be recommended in these cases.

In our series, eight episodes of nephrotoxicity were reported in the early post-transplant period which were characterized by an increase in creatinine levels above the upper reference limit of the test. During these episodes of renal insufficiency, as well as during the stable course of renal function no significant difference was observed between the cyclosporin levels measured by both immunoassay techniques, i.e. mFPIA and EMIT® (Fig. 5). In these cases, the adjustment of CsA dosage was the same as if CsA levels had been measured either by mFPIA or EMIT®.

CsA monitoring has been recommended to reach a therapeutic level, but also to avoid drug toxicity such as nephrotoxicity and hepatotoxicity. Given the present level of knowledge, there is insufficient reason to consider that CsA metabolites might play an important part in treatment activity and toxicity. In terms of therapeutic follow-up this confirms that non specific CsA assays are not of major interest and if further information is required in specific cases, e.g. in liver or heart transplant recipients, it would be suitable to determine selected metabolites specifically. Our results confirm the lower specificity of the monoclonal antibody used in mFPIA as compared to the one used in EMIT®. The use of a higher reference range of cyclosporin blood levels might not overcome this deficiency since a high variation in the overestimate of cyclosporin parent compound in populations of heart and liver transplants has been demonstrated. From the point of view of nephrotoxicity this study showed similar results for both EMIT® and mFPIA.

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