Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 1, pp. 43–48, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Pharmacokinetics of cyclosporin A during pregnancy; monitoring of treatment and specific assays of cyclosporin, based on five liver transplant patients

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Abstract: Very little information is available concerning the pharmacokinetic behaviour and monitoring of cyclosporin A (CsA) during pregnancy, notably after liver transplant. Monitoring of blood levels of CsA is considered to be one of the best tools for evaluation of the efficacy of immunosuppressant treatment. The aim of this study was to bring together new information concerning pregnant women receiving immunosuppressant treatment with CsA and, in view of the special pathophysiological status of such patients, to compare pharmacokinetic profiles of changes in blood levels of CsA and of the combination of CsA plus metabolites. Specific (CsA-S kit) and a new non-specific (CsA-NS kit) assays of CsA were carried out in five hospitalized pregnant patients who had received liver transplants between the 6th and the 41st weeks of amenorrhea. The results of five cases investigated lead to the following conclusions: (1) The pharmacokinetic behaviour of native CsA in the pregnant woman between the 6th and 41st weeks of amenorrhea suggests no systemic accumulation nor any radical need for changes in dosage schedule as compared with a non-pregnant patient. (2) Monitoring based upon simultaneous use of the CsA-NS and CsA-S kits may be a source of analytical bias and hence confusion for the physician. (3) Determination of an experimental CsA-NS/CsA-S accumulation ratio (based upon analysis of single concentrations or processing of AUCs) is of interest only if specific assays involve not only CsA itself but also its principal metabolites. (4) Monitoring based upon single measurements of residual CsA levels only, is necessary and adequate. Furthermore, such an approach is less costly. (5) The new CsA-S kit suggested by Abbott Laboratories provides satisfactory results and is well suited to real time monitoring, which is needed by the physician in hospital practice. Obviously, the majority of these remarks are also valid at times other than during pregnancy.

Keywords: *Cyclosporin; pregnancy; drug monitoring; pharmacokinetics; liver transplantation.*

Introduction

Very little information is available concerning the pharmacokinetic behaviour and monitoring of cyclosporin A (CsA) during pregnancy, notably after liver transplant. At any event, physicians attempt to adjust the immunosuppressant dosage schedule on the basis of data relative to gestational age, the type of transplant and the individual susceptibility of patients. In two earlier studies devoted to liver transplantation during pregnancy, the authors reported the existence of transplacental penetration of CsA during the third trimester as well as an accumulation of CsA in products of conception during the first trimester [1, 2].

Monitoring of blood levels of CsA is now considered to be one of the best tools for evaluation of the efficacy of immunosuppressant treatment. It enables reduction in the incidence of iatrogenic effects specifically related to CsA while at the same time maintaining an acceptable level of immunosuppression [3–5]. Thus monitoring whole blood has been recommended rather than serum, provided that a specific method is used, capable of exclusively quantifying CsA and not its metabolites [3–5]. Analytical techniques used involved either high-performance liquid chromatography (HPLC) or radioimmunoassay (RIA) [6, 7]. Although very effective, these methods are laborious and are relatively unsuitable for routine analysis. Several RIA tests using specific monoclonal antibodies, the crossover reactivity of which with metabolites of CsA is very slight, are currently available on the market or are in the process of development [8–10]. Several years ago, Abbott

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Laboratories marketed an automated assay technique using immunoanalysis by fluorescence polarization (FPIA = fluorescence polarization immunoassay) with a TDx® multiparametric analyser. The antibody used is in fact non-specific (CsA-NS kit) and, as will be seen, has considerable crossover reactivity with the prinicipal metabolites of CsA [9]. Recently, a selective antibody of native CsA has been developed by Abbott Laboratories (CsA-S kit) [10]. Whether concerning specific radioimmunoassay or the specific determination of CsA by FPIA, correlation with measurements by HPLC is now excellent [10]. It is clear that the value of specific assay methods is to take into account only the native compound which contains the maximum immunosuppressant activity.

The aim of this study was to bring together new information concerning pregnant women receiving immunosuppressant treatment with CsA and, in view of the special pathophysiological status of such patients, to compare pharmacokinetic profiles of changes in blood levels of CsA and of the combination of CsA plus metabolites. In all cases, studies involved patients with a stable metabolic status. Specific (CsA-S kit) and non-specific (CsA-NS kit) assays of CsA were carried out in five hospitalized pregnant patients between the 6th and the 41st weeks of amenorrhea.

Experimental

Patients

Five pregnant women (W_1–W_5) having received an orthotopic liver transplant were included in this study. Pregnancy occurred 22 (W_1), 21 (W_2), 13 (W_3), 23 (W_4) and 13 (W_5) months, respectively, after surgery. Patients W_1, W_2 and W_5 were hospitalized at term while patient W_3 was hospitalized after 6 weeks of amenorrhea in order to undergo a termination of her pregnancy. This pregnancy has been confirmed by ultrasonography. Patient W_4 had abdominal pain without obvious signs of infection nor of the premature onset of labour. Patients gave their written and informed consent for a series of peripheral blood samples to be drawn for study of the profile of pharmacokinetic changes involving CsA and its principal metabolites. During pregnancy, immunosuppression was obtained using the following therapeutic combination: CsA, prednisone and azathioprine administered orally. Table 1 shows morphometric and laboratory data (mean ± standard error of mean), clinical history and characteristics of the immunosuppressant treatment of the five patients at the time of the study.

Experimental and analytical procedure

During the period of hospitalization, laboratory and in particular clinical chemistry parameters were monitored every 2 days [ASTRA 8® analyser (Beckman, Gagny, France) and COBAS® analyser (Roche Neuilly, France)].

Blood samples were drawn by a cannula at times during two successive oral administrations of CsA. The first sample was obtained immediately before the next dose of CsA in order to determine the blood level. Because of dosage schedules specific to each patient (two or three doses per day), subsequent blood samples were drawn at times 1, 2, 3, 4, 6 and 8 h after oral administration of Sandimmum® in W_3, W_4 and W_5. In patients W_1 and W_2, an additional sample was drawn 12 h after administration. CsA levels were measured using a TDx" multiparametric analyser (Abbott Laboratories, North Chicago, IL, USA) by both the specific and the non-specific techniques [11, 13]. Samples were analysed immediately. Limits of detection for the two assay techniques were 25 $\mu g\ l^{-1}$ and 50 $\mu g\ l^{-1}$ for the CsA-S kit and the CsA-NS kit, respectively.

In the case of the CsA-NS kit, crossover reactivity (%) of the principal metabolites (M) with CsA is 38.9, 34.1, 105.0, 70.4 and 87.4 for M-1 (AM-9), M-8, M-17 (AM-1), M-21 (AM-4N) and M-18, respectively [11, 14]. There is negligible crossover reactivity (<1–2%) between other metabolites of CsA and the parent compound. With the CsA-S kit, crossover reactivity of the principal metabolites with CsA is 15.3, 5.3, 8.2 and 3.7 for M-1 (AM-9), M-8, M-17 (AM-1) and M-21 (AM-4N), respectively (there is no crossover reactivity with M-18) [13].

Kinetic evaluation

Pharmacokinetic analysis involved an independent compartmental model method. The area under the curve of concentrations (AUC_{0-T}) was determined by the trapezoidal rule method between time 't = 0' (first sample) and time 'T' of the last sample. An experimental accumulation ratio (R_{exp}) was routinely calculated between (AUC_{0-T}) CsA-

Table 1
Morphometric and biological data, gestation state and immunosuppressive treatment of the five patients

	Patient				
	W_1	W_2	W_3	W_4	W_5
Age (years)	33	24	33	21	27
Weight (kg)	67	64	79	63	59
Parity	G3P3	G2P1	G5P4	G1P0	G1P1
Total serum proteins (65–80 gm l^{-1})	60	63	66	64	62
Serum urea (2.5–7.5 mmol l^{-1})	4.8	3.7	4.2	5.0	6.5
Creatine (45–105 μmol l^{-1})	4.8	58	63	52	71
ALT Alamine transaminase (<30 IU l^{-1})	32	25	17	20	26
AST Aspartate transaminase (<40 IU l^{-1})	32	31	14	30	36
Total serum bilirubin (<17 μmol l^{-1})	8	12	15	10	13
Term at day of the study	39 2/7 (delivery day)	38 2/7 (delivery day)	6 3/7 (induced abortion)	34 1/7 (abdominal pain)	37 2/7 (delivery day)
Orthotopic transplant	liver	liver	liver	liver	liver
Hepatic disease	autoimmune cirrhosis	fulminant hepatitis	autoimmune cirrhosis	fulminant hepatitis	autoimmune cirrhosis
Transplantation date	August 1988	July 1988	January 1990	September 1989	January 1990
CsA daily dose	100 mg × 2	80 mg × 2	90 mg × 3	90 mg × 3	90 mg × 3
Prednisone daily dose (mg)	10	14	10	10	10
Azathioprine daily dose (mg)	50	50	50	50	—

Normal laboratory values are shown in brackets.

Table 2
Individual pharmacokinetic parameters of the five patients

	Patient									
	W_1		W_2		W_3		W_4		W_5	
	CSA-NS*	CSA-S†	CSA-NS	CSA-S	CSA-NS	CSA-S	CSA-NS	CSA-S	CSA-NS	CSA-S
AUC_{0-T} (μg h^{-1} l^{-1})	1211	955	1755	1221	2065	2525	1488	1171	1758	1219
Delta AUC_{0-T} (%)	21.1		30.4		18.2		21.3		30.6	
R_{exp}‡	1.27		1.44		0.82		1.27		1.44	
CL/F (ml min^{-1} kg $F\%^{-1}$)	20.5	26.1	11.9	17.1	9.2	7.51	13.6	17.3	14.4	20.8
MRT_{0-T} (h)	4.59	4.45	4.95	5.21	3.75	3.70	2.91	3.02	3.43	3.25
C_{max} at plateau (μg l^{-1})	172	141	245	178	489	620	326	266	230	175

*Non-selective antibody.
†Selective monoclonal antibody.
‡Experimental ratio: [AUC_{0-T} CsA-NS/AUC_{0-T} CsA-S].

NS and AUC_{0-T} CsA-S in order to identify relative proportions of CsA and of its principal metabolites. The mean residence time (MRT; h) was calculated using the formula suggested by Yamaoka *et al.* [15]. Total clearance (CL/*F*; ml min^{-1} kg^{-1}) was calculated using the formula CL/F = dose/AUC_{0-T}, an expression in which *F* (%) represents the oral bioavailability of the drug. Finally, C_{max} and C_{min} were determined experimentally.

Results and Discussion

Acceptability of immunosuppressant treatment remained excellent and no episode of rejection or of worsening of liver function were seen during the time of pregnancy. Figure 1 shows for each patient comparative changes in blood levels of CsA and metabolites (CsA-NS) and CsA (CsA-S) in relation to time. At the plateau, fluctuations in levels of CsA-S and of CsA-NS were slight. In W_1, W_2, W_4 and W_5, CsA-NS levels were invariably higher than CsA-S levels. The opposite applied in patient W_3. It is important to note that in this last woman concentrations of drug were invariably greater than those in other patients. Table 2 shows individual pharmacokinetic data of the patients. In W_1, W_2, W_4 and W_5, AUC_{0-T} were similar regardless of the treatment regimen, this giving an R_{exp} of 1.27 or more. In contrast, R_{exp} was less than 1 in patient W_3. As a result, by first analysis MRT and the CL/*F* of CsA appeared to be decreased in this patient.

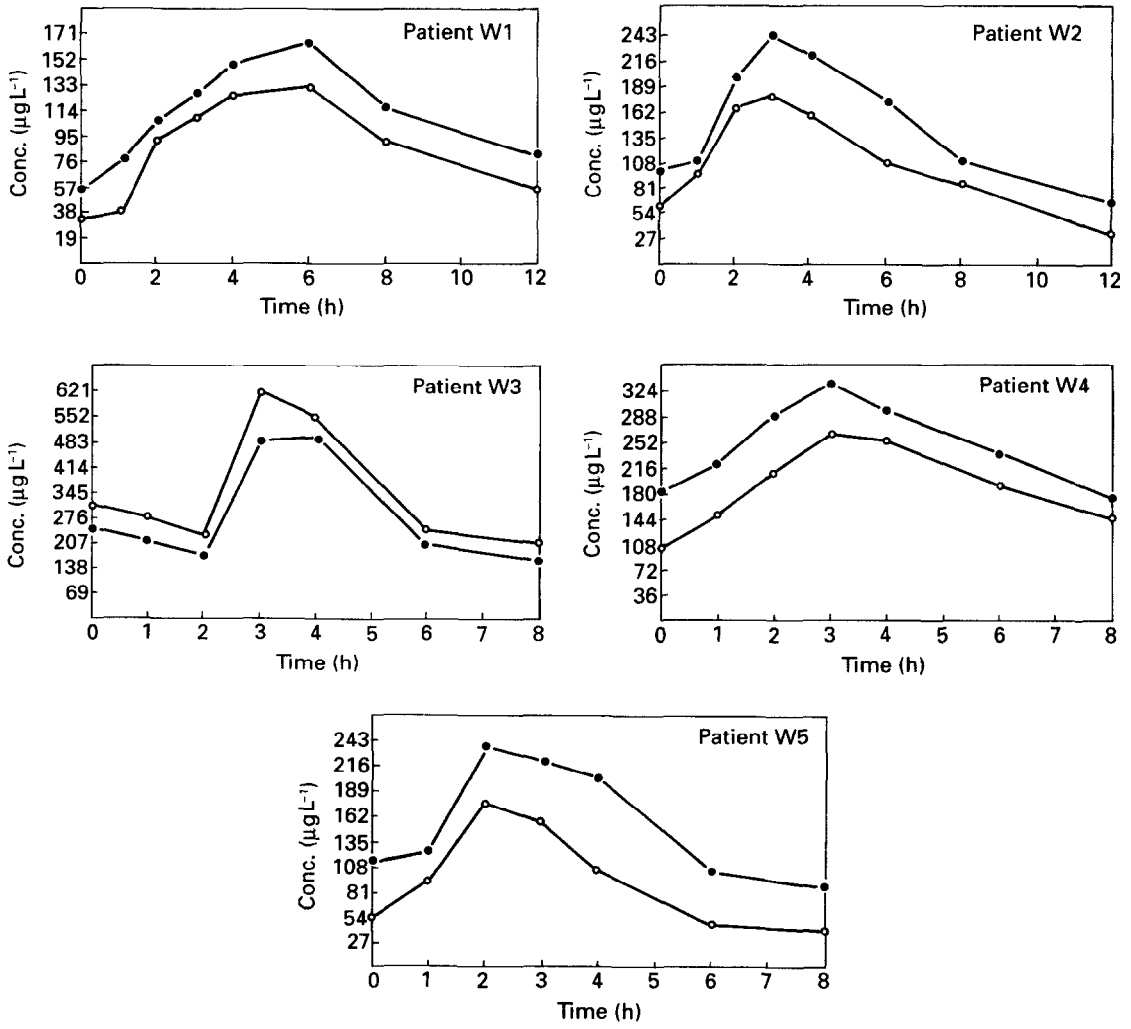

Figure 1
Comparison of maternal profiles of CsA-NS (●) and CsA-S (○) plasma levels at plateau over time after oral administration for each patient (n = 5).

All patients had well controlled immunosuppressant treatment and fluctuations in CsA levels remained slight. Among recommendations contained in Consensus Reports on CsA monitoring, emphasis is placed upon the value of specific measurement as well as of the need to make measurements using whole blood [3–6]. It is impossible to determine the specific concentration of CsA in plasma when only a non-specific measurement made in whole blood is available. Generally speaking, no reliable extrapolation is possible from a single measurement. It is for this reason that it was attempted to examine the kinetic profiles of CsA and of CsA plus metabolites over a dosage interval. Information provided by measurement of native CsA and of the CsA plus metabolites group may be analysed on the basis of the following features:

- The specific assay method, recently suggested by Abbott Laboratories, is the first specific automated technique for CsA. Like all techniques specific to native CsA, it is of value in that metabolite M-17 (AM1) is often found in larger amounts than CsA in the blood of treated patients [13]. However, if any particular metabolite is considered as having clinical activity and/or a significant toxic potential, it might be considered appropriate to carry out a specific assay for that metabolite. In practice, it is difficult and probably of no value to undertake pharmacokinetic studies of this type. CsA being exclusivcely metabolized by the liver and its total clearance being represented by hepatic clearance, the degree of its metabolism is known to be subject to multifactorial influences, in particular in transplant patients.
- Certain published results will be in favour of simultaneous determination of CsA and of the CsA plus metabolites group. It is accepted that the existence of crossover reactivity between CsA and its principal metabolites raises no particular problem when the CsA-NS/CsA-S ratio of concentraitons or AUC remains relatively constant (of the order of 1.25). This has been shown in particular in renal transplant patients and was indeed what was found in four of our own cases [14]. It has also been shown following heart or liver transplant, that during the immediate post-transplant period blood levels of metabolites of CsA are often higher if assay is by FPIA rather than by HPLC [6, 9].
- Patient W_3 had an R_{exp} of less than 1, indicating that circulating levels of CsA-S were constantly higher than those of CsA-NS. Such a result is illogical and led to uninterpretable MRT and CL/F values in this patient. As has already been mentioned above, the polyclonal antibody used in the CsA-NS kit has a high level of crossover reactivity with the principal metabolites of CsA [9]. This result is probably not indicative of graft dysfunction but, possibly due to an analytical artefact, e.g. a blood component interfering with metabolites binding to antibody.

It must be pointed out that the five patients of the study are always followed in our centre. At the present time, their immunosuppressant treatments are similar to those they had during the study. In all cases no accumulation of CsA is noted and the tolerability is excellent. Thus, it was verified that patient W_3 is always presenting the special analytical profile described above although her therapeutic schedule remains unchanged.

The five cases reported lead to the following conclusions: (1) The pharmacokinetic behaviour of native CsA in the pregnant woman between the 6th and 41st weeks of amenorrhea suggests no systemic accumulation nor any radical need for changes in dosage schedule as compared with a non-pregnant patient (obviously the impact of CsA and of its metabolites on foetal development still remains to be defined). (2) Monitoring based upon simultaneous use of the CsA-NS and CsA-S kits may be a source of analytical bias and hence confusion for the physician leading to unnecessary modifications of treatment which is in fact well controlled. (3) Determination of an experimental CsA-NS/CsA-S accumulation ratio (based upon analysis of single concentrations or processing of AUCs) is of interest only if specific assays involve not only CsA itself but also its principal metabolites (thus in practice by HPLC). (4) Monitoring based upon single measurements of residual CsA levels only, is necessary and adequate. Furthermore, such an approach is less costly. (5) The new CsA-S kit suggested by Abbott Laboratories provides satisfactory results and is well suited to real time monitoring, which is needed by the physician in hospital practice. Obviously, the majority of these remarks are also valid at times other than during pregnancy.

In summary, it is emphasized that overall review of clinical, laboratory and pharmacological data is essential before envisaging any

change whatsoever in the immunosuppressant treatment regimen.

References

[1] P. Bourget, H. Fernandez, H. Bismuth and E. Papiernik, *Transplantation* **49**, 663–664 (1990).
[2] P. Bourget, H. Fernandez and C. Delouis, *Transplantation* **49**, 1306–1307 (1991).
[3] L.M. Shaw, L.D. Bowers and L. Demers, *Clin. Chem.* **33** (Special Report), 1269 (1987).
[4] L.M. Shaw, R.W. Yatscoff and L.D. Bowers, *Clin. Chem.* **36**, 1841–1842 (1990).
[5] B.D. Kahan, L.M. Shaw, D. Holt, J. Grevel and A. Johnson, *Clin. Chem.* **36**, 1510–1514 (1990).
[6] L.M. Shaw, *Clin. Chem.* **35**, 1299–1300 (1989).
[7] W. Niederberger, P. Schaub and T. Beveridge, *J. Chromatogr.* **182**, 454–458 (1980).
[8] K.R. Copeland and R.W. Yatscoff, *Ther. Drug. Monit.* **10**, 453–456 (1988).
[9] D.E. Sgoutas and M. Hammarstrom, *Transplantation* **47**, 668–671 (1989).
[10] P.E. Ball, H. Munzer, H.P. Keller, E. Abish and J. Rosenthaler, *Clin. Chem.* **34**, 257–260 (1988).
[11] A.J. Pesce, T.J. Schroeder and M.R. First, *Transplant. Proc.* **22**, 1171–1173 (1990).
[12] P. Wang, V. Meucci and E. Simpson, *Transplant. Proc.* **22**, 1186–1188 (1990).
[13] R.W. Yatscoff, K.R. Copeland and C.J. Faraci, *Clin. Chem.* **36**, 1969–1170 (1990).
[14] G.L. Lensmeyer, D.A. Wiebe and I.H. Carlson, *Transplant. Proc.* **20** (suppl 2), 614–622 (1988).
[15] K. Yamaoka, T. Nakagawa and T. Uno, *J. Pharmacokin. Biopharm.* **6**, 547–558 (1979).

[Received for review 2 January 1992; revised manuscript received 26 March 1992]