

THERAPEUTIC DRUG MONITORING OF CYCLOSPORINE IN TRANSPLANTED PATIENTS. POSSIBILITIES, CONTROVERSY, CAUSES FOR FAILURE

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Abstract

Monitoring cyclosporine therapy is required by the narrow therapeutic index, high pharmacokinetic variability, the increased risk of drug interactions CYP 3A4-dependent and nephrotoxicity of the substance substance. The controversies raised by clinical practice are the best time of sampling, the analytical methods used and the feasibility of the pharmacokinetic models to estimate the useful concentrations for preventing rejection and nephrotoxicity, and inherent pharmacokinetic drug interactions with concomitant treatments.

Rezumat

Monitorizarea terapiei cu ciclosporină este impusă de indicele terapeutic îngust, variabilitatea farmacocinetică foarte mare, riscul crescut de interacțiuni medicamentoase CYP 3A4-dependente și de nefrotoxicitatea substanței. Controversele pe care le ridică practica clinică sunt momentul optim al prelevării probei, metodele analitice utilizate și fezabilitatea modelelor farmacocinetice în condițiile estimării concentrațiilor utile pentru prevenirea rejecției și a nefrotoxicității, dar și a administrării de tratamente concomitente cu interacțiuni medicamentoase farmacocinetice inerente.

Keywords: cyclosporine, transplantation, therapeutic drug monitoring

Introduction

Cyclosporine, a cyclic peptide composed of 11 aminoacids, is a selective immunosuppressant agent, acting by binding to cytosolic protein – cyclophilin from the immunophilins class and the formed complex inhibits calcineurin, preventing the activation of Th cells and production of IL2. It became the main immunosuppressive agent used in transplant patients

Although indispensable post-transplant, cyclosporine presents some pharmacokinetic and pharmacotoxicologic features that make pharmacotherapy monitoring imperative:

- narrow therapeutic index;
- extremely high pharmacokinetic variability;

- differences in bioavailability between different pharmaceutical products on the market;
- high risk of interactions CYP 3A4 and CYP 3A5 dependent with direct and immediate consequences (over/underdosing);
- nephrotoxicity dose-dependent.

Summary data on the pharmacokinetics of cyclosporine

Cyclosporine has a variable oral absorption both intra- and interindividual (on average approx. 30%), plasma peak is 1-3 h after administration (pharmaceutical formula such as microemulsion formulations have a slightly better bioavailability and less variable). Distribution is large (V_d approx. 13 l/kg), and significant protein binding (about 90%, mainly lipoproteins) also binds to the figurative elements (red blood cells). Metabolism, including hydroxylation and N-methylation) is CYP 3A-dependent. Both inducers and inhibitors of CYP 3A4 are likely to significantly alter the blood concentration of cyclosporine. Many metabolites (over 30), with reduced activity or lack of immunosuppressive action) are eliminated mainly by bile, parenteral drug half-life is approx. 6 h. [2, 18, 19]

Methods for therapeutic monitoring. Controversies

The main challenges of monitoring cyclosporine therapy are:

1. What is the best time of blood sampling and which is the biological matrix that is most appropriate?

Since the introduction of cyclosporine in post-transplant therapy, recommendations and guidelines for practice were continually modified [6, 7, 15], but the most difficult problems of therapeutic monitoring remain unresolved.

Monitoring should take into account the blood level of cyclosporine and the therapeutic interval (different for renal, liver and heart transplantation) and the correlation that exists between this interval and acute graft rejection, on one hand, and nephrotoxicity, on the other hand.

Because cyclosporine binds significantly to figurative elements, especially to red blood cells, whole blood is a better biological matrix for assessing cyclosporine concentration than plasma. Any concentration value issued by a laboratory must specify the matrix used (whole blood/plasma) and the analytical method used (HPLC, RIA, etc.)

The purpose of monitoring is to prevent rejection (graft survival) and improved tolerance (avoidance of adverse reactions, particularly nephrotoxicity and too high immuno-suppression). C_{through} residual

concentration (C_0 -through peak, pre-dose concentration) is directly correlated with nephrotoxicity, but it is not a useful marker for prediction of acute rejection. Instead, both nephrotoxicity and acute rejection are better correlated with the area under the concentration-time curve measured between 0 - 4 h or 0 -12 h (AUC_{0-4} , AUC_{0-12}). These values can be better estimated using the value of C_2 (blood level 2 h post-dose) than the residual concentration (C_0). This is the consensus reached by experts. Clinical benefit is estimated by the absence of rejection and no increase in serum creatinine. A retrospective study on 168 patients with kidney transplant [8] showed that most patients with value C_0 in the recommended limits were below the target value of C_2 required by guides ($<1500 \pm 20\%$ mg/L at 2 months after renal transplantation in 68% of patients, respectively $<800 \pm 20\%$ mg/L in 55% of patients after one year) that would have required a dose increase with 40% in the first week post transplant and with 50% in treatment maintenance to comply with the limits imposed by the guidelines.

Target values for C_0 , C_2 respectively for various types of transplantation are presented in Table I [2, 13]. Frequency of cyclosporine blood levels determination should be at 2-3 days (in the first 4 weeks post-transplant), then monthly after 3 months.

Table I
Target monitored values for blood levels

No. crt.	Transplant type	Target values (whole blood)		Observations
		C_0 (ng/mL)	C_2 (mg/L)	
1	Renal	200-400	1.4-2	15 days post-transplant
		125-275	1.2-1.8	2-3 months post-transplant (good renal function)
		100-125		2-3 months post-transplant (poor renal function)
		100-150	0.7 -1	6 months – 1 year post-transplant
		75-150		> 1 year post-trasplant
2	Hepathic	250-350	0.8-1.2	First 6 months
		100-200	0.6 -1*	6 months – 1 year post-transplant
3	Cardiac	250-350		initially
		100-200	0.3-0.6	6 months – 1 year post-trasplant

* Other authors suggest other targets: two therapeutic ranges have been proposed C_2 high (0.7 to 1 mg/L) and C_2 reduced (0.3 to 0.6 mg/l) with C_0 of 100-200 ng/ml [13].

2. What are the pharmacokinetic interactions between different immunosuppressive drugs administered concomitantly?

Target cyclosporine blood values imposed by the guidelines for monitoring were obtained in the context of post-transplant co-administration of more immunosuppressive drugs (cyclosporine associated with corticosteroids and mycophenolate mofetil or azathioprine and also sirolimus can be added). Other combinations require reassessment of the

optimal concentration. Cyclosporine - tacrolimus association is not recommended (additive nephrotoxicity). In addition, cyclosporine reduces *in vitro* hepatic clearance of tacrolimus with values between 61-70% [5].

Sirolimus is metabolized by CYP 3A4/5. Although CYP 3A5 has a higher affinity for sirolimus than CYP 3A4, its hydroxylation undergoes 3 times faster by CYP3A4, to which cyclosporine has higher inhibitory capacity. Consequently, hepatic clearance of sirolimus is diminished (*in vitro* studies) with approx. 44% in the presence of cyclosporine. These aspects must be taken into account when co-administering the two substances. [17]

3. What are the CYP 3A4-dependent interactions with clinical implication that influence monitoring of cyclosporine therapy?

Cyclosporine is extensively metabolized by CYP 3A isoforms (CYP 3A4 and CYP 3A5) with the formation of more than 30 metabolites. Since their separation and identification is difficult using different analytical methods, a nomenclature consistent with the positioning of the compound oxidation by consensus groups was required [6]. Although polymorphism of CYP 3A4/5 is not investigated by routine CYP 3A4 is involved in the metabolism of almost 50% of known drugs and is easily susceptible to induction and enzyme inhibition, respectively [16].

Immunosuppressive medication frequently promotes fungal infections which are compulsorily treated. Concomitant treatment with cyclosporine and ketoconazole or fluconazole (antifungal imidazoles with important inhibitory capacity on CYP 3A4 at usual doses) marked increases cyclosporine blood levels requiring dosage adjustment in clinical practice by using C_2 and C_0 values. Prediction models are available for pharmacokinetic drug interactions which estimate the blood value of the drug (including cyclosporine) using suspensions of human hepatocytes and phenotypic data on the P450. [9, 10, 11]

Bacterial infections in patients under immunosuppressive treatment should be treated quickly, but macrolides (especially erythromycin) should be avoided because of the marked increase of cyclosporine blood levels. Also quinopristine, dafopristine and pristinamycine have CYP 3A4 inhibitory activity and require reassessment of cyclosporine dosage. On the other hand, antibacterial alternative treatment that do not pharmacokinetically interact with cyclosporine may lead to additive nephrotoxicity that must be evaluated in the context of therapy clinical safety (aminoglycosides, sulfonamides-trimethoprim). Enzyme inducers (rifampicin used for tuberculosis reactivation after immunosuppression)

could lead to reassessment of cyclosporine dose (increasement) and a clinical and histological closer supervision [2].

In addition, the possibility of cytomegalovirus infection due to immunosuppression requires prophylactic administration of ganciclovir or valganciclovir [12, 20], in which case monitoring the therapy with both cyclosporine and with the antiviral used is recommended.

In the context of poly pathology, other medicines with concomitant enzyme inhibitory effect (calcium channel blockers, amiodarone) may determine re-evaluation of the treatment with cyclosporine on analytical determination bases, with a corresponding reduction of dosage.

As a substrate for CYP 3A4, cyclosporine may interfere with competitive enzymatic inhibition. But in turn, cyclosporine has inhibitory activity on CYP 3A4 (4 times higher than tacrolimus), it can inhibit metabolic reactions such as nifedipine oxidation, testosterone 6 β -oxidation, demethylation of diazepam, of terfenadine and erythromycin, midazolam and triazolam hydroxylation. Cyclosporine is also, a substrate for P-glycoprotein (ABCB1), whose role in drug transport and efflux is initiated by MDR1. These pharmacokinetic issues may lead to clinically relevant drug interactions [14].

4. Which is the most appropriate analytical method to monitor therapy (TDM) with cyclosporine?

Although HPLC has been and remains the reference method in monitoring pharmacotherapy, most analytical methods used for determining cyclosporine in the blood are immunoassay methods (RIA - *radioimmunoassay*, respectively FPIA - *Fluorescence polarized immunoassay*), which are simple, fast, easy to implement and are widely used in laboratories. The first immunoassay methods used were non-selective and they didn't discriminate between cyclosporine and its metabolites, so the results of analytical determinations always overestimated parental drug concentration. Even newer methods considered "specific" and selective using monoclonal antibodies (compared to the original methods based on non-specific polyclonal antibodies) lead to inconsistent results compared to HPLC determination, considered the reference method. Discordant results are always over-concentration of cyclosporine compared to the results obtained by processing the same sample by HPLC, because metabolites cross reactivity [1]. In addition, when using the value of C_2 for monitoring, samples should be diluted to be analyzed by RIA in order to be in the range of linearity. The problem of size difference and clinical significance remains controversial, but pharmacokinetic processing of analytical data leads to large discrepancies in values obtained using

immunoassay techniques vs. HPLC. Although this fact was reported long ago [1], recent studies show that the AUC_{0-12} and C_{max} values obtained from immunoassay vs HPLC are significantly higher [4]. Direct consequence of this is inadequate therapeutic monitoring of cyclosporine using C_2 and AUC values, if the same assay using the same laboratory is not always used for the same patient.

Although there are pharmacokinetic population models in routine monitoring of cyclosporine based on Bayesian estimation method which attempts to identify sources of inter-and intraindividual variability (such as for candidates for kidney transplantation in pediatric practice, where significant covariates were body weight, serum creatinine, hematocrit and serum total cholesterol) [3], difficulties in obtaining a suitable model remain high given the considerations mentioned above.

Conclusions

Therapeutic monitoring of cyclosporine in transplant patients is a useful tool in adjusting drug dosage to prevent acute rejection, respectively nephrotoxicity and predictable dose-dependent adverse reactions. Literature data show that determining the value of C_2 (blood concentration 2 hours post dose) is more useful than to measure residual concentration predose (C_0). Calculation of pharmacokinetic parameters, pharmacokinetic models and dosage adjustment should take account of inter-and intraindividual variability, co-administered treatments and that target concentrations values are obtained by immunoassay techniques. For a particular patient is imperative necessary cyclosporine dosage by the same method by the same laboratory, otherwise dosage adjustments can often lead to over/underdosing with adverse clinical consequences.

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