Calcineurin inhibitors in lupus nephritis

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Maintenance immunosuppressive treatment of lupus nephritis is aimed at preventing flares and maintaining stable renal function. The combination of low-dose glucocorticoids and a purine synthesis inhibitor (azathioprine or mycophenolate) is currently considered as the standard of care. This therapeutic strategy improves clinical outcomes in patients with lupus nephritis, however it may not be sufficient to completely abrogate proteinuria in some cases. In addition, albeit displaying a favourable safety profile, such treatment may be burdened by gastrointestinal side-effects, hematologic toxicity and severe infections.

Calcineurin inhibitors (CNI) also proved effective and well tolerated in the mid-term for lupus nephritis [1, 2]. In this issue, Argolini et al. compared the effects of cyclosporine (CsA), mycophenolate (MPA) and azathioprine (AZA) as maintenance treatment in 106 patients with lupus nephritis. The authors found no differences among treatments in maintaining stable kidney function and complete remission, although patients treated with CsA had significantly higher baseline proteinuria. After 8 years of follow-up, the authors observed a trend towards reduced frequency of renal flares in patients given CsA compared to those treated with MPA or AZA, even though the difference did not reach statistical significance. No difference in the number and severity of side effects was demonstrated among groups [3]. These data are intriguing and may spark renewed interest for the use of CNI in the long-term maintenance treatment of lupus nephritis, although concerns remain about their potential toxicity. A good knowledge of pharmacologic characteristics of CNI and their interactions with other drugs may considerably reduce the risk of side effects.

Calcineurin is a phosphatase composed by a 61-kDa calmodulin-binding catalytic subunit (calcineurin-A), and a 19-kDa calcium-binding regulatory subunit (calcineurin-B). This protein participates in a wide array of cellular processes and calcium-dependent signal transduction pathways, including T-cell activation. Calcineurin is similarly inhibited by CsA and tacrolimus (TAC), despite their different chemical structures (CsA is a cyclic endecapeptide, TAC is a macrocyclic lactone). CsA and TAC immunosuppressive properties rely on the downstream inhibition of IL-2 signaling, ultimately interfering with T-cell activation, proliferation and differentiation (Fig. 1). Beyond their immunosuppressive activity, CNI may also exert direct protective effects on podocytes, such as the inhibition of calcineurin-induced dephosphorylation of synaptopodin, a critical protein that regulates actin filaments of the podocyte cytoskeleton [4]. Moreover, CNI can also improve podocyte viability and reduce their migrating activity [5].

Because of these beneficial effects, CNI have been successfully used in different types of kidney diseases. However, their use may be burdened by serious side-effects, including nephrotoxicity, hypertension, diabetes, neurologic complications, dyslipidemia, infection, cutaneous and gastrointestinal disorders. Interestingly, most of these side effects depend on CNI doses or drug-to-drug interactions.

Different transporters and metabolic pathways are responsible for the absorption and metabolism of CNI. P-glycoprotein (P-gp), an ATP-dependent pump which regulates the uptake of CNI and other drugs, and cytochrome P450 (CYP450) enzymes such as CYP3A4, which oxidize toxins and drugs, are particularly relevant in this context. Since genetic variants in P-gp or CYP450 can alter CNI handling and favor nephrotoxicity, genetic testing prior to treatment initiation could theoretically help predicting the optimal drug dose. However, such an approach would be too expensive and time-consuming to adopt in clinical practice.

In the absence of a reliable direct assessment of CNI metabolism, the characteristics of the patient to be treated should be the first step when deciding the initial CNI dosage. In this context, it is important to consider factors that

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Fig. 1 Mechanism of action of cyclosporin, tacrolimus, azathioprine and mycophenolate. Calcineurin inhibitors (CNIs) are lipophilic prodrugs that bind to specific cytoplasmic receptors termed immunophilins. These receptors include cyclophilin (CyP) and FK-binding protein (FKBP), which bind cyclosporin (CsA) and tacrolimus (TAC) respectively. CNIs inhibit the activity of calcineurin, a complex of phosphatases activated by the intracellular calcium released after T-cell receptor (TCR) engagement and co-stimulation from antigen-presenting cells (APC). Calcium-bound calcineurin dephosphorylates a family of proteins called nuclear factor of activated T cell (NFAT), which participate to the transcriptional activation of genes that encode interleukin-2 (IL-2) and other proinflammatory cytokines.

IL-2 and other cytokines such as IL-15 bind to their receptors, which activate a cascade of kinases that eventually propagate the signal for T-cell proliferation and differentiation. The interaction of co-stimulatory molecules (CD40/CD40L and B7/CD28) promotes the activation of kinases and transcription factors (e.g. JAK, PI-3 K, mTOR, STAT) that further enhance T-cell division and clonal expansion. Therefore, CNIs chiefly suppress the immune response by down-regulating the transcription of various cytokine genes, of which the most significant is IL-2. By contrast, azathioprine (AZT) and mycophenolic acid (MPA) mechanism of action consists in blocking de novo purine synthesis, thus inhibiting T-cell proliferation

may inhibit CYP450 enzymes and cause CNI accumulation and toxicity, such as old age, morbid obesity and the use of imidazoles, macrolides, quinolones, and some calcium channel blockers. On the other hand, CYP450 inducers (childhood, malnourishment, anti-epileptic drugs, oxacillin, nafcillin, rifampicin) can increase the catabolism of CNI, thus reducing their activity. In elderly or obese individuals, CNI standard doses should be reduced; conversely the initial dose should be higher in children and in malnourished individuals. Despite these well-known facts, the impact of age and body weight in CNI-treated patients is often neglected in clinical studies.

Monitoring of CNI blood trough levels and area-under-the curve pharmacokinetics have been advocated as strategies to define the optimal dosage, in order to maximize the therapeutic effect and avoid toxicity. However, the relevance of therapeutic drug monitoring with these methods is limited by the large intra- and inter-patient variability, which severely limits the accuracy of pharmacokinetic models and bioavailability estimates. As a matter of fact,

no correlation has been found between CNI blood levels and their pharmacologic effects in the field of kidney transplantation [6]. The implicit assumption of therapeutic drug monitoring that CNI blood levels directly reflect their intracellular (i.e. pharmacologically active) concentration may be frequently illusory and sometimes misleading. For instance, low CNI blood levels may be associated with elevated intracellular concentrations in case of a congenital deficits of P-gp, which actively removes CNI from cells. Similar effects can be observed with the concomitant use of P-gp antagonists such as colchicine, quinidine, protease inhibitors and clotrimazole, which reduce CNI efflux and increase their intracellular accumulation, predisposing to toxicity despite low blood levels [7]. On the other hand, lymphocytes stimulated by IL-2 may trigger a P-gp-mediated multidrug resistance in patients with lupus nephritis, which can be further enhanced by P-gp inducers such as certain anti-epileptic drugs (carbamazepine, phenobarbital, phenytoin), rifampin, tenofovir, nefazodone, trazodone, prazosin and doxorubicin. This could lead to

Table 1 General guidelines for the use of CNI as maintenance treatment in patients with lupus nephritis

Avoid CNI in patients at high risk of renal toxicity (eGFR < 40 mL/min/1.73 m ² , tubulo-interstitial fibrosis, diabetes, hypertension)
CNI could be particularly useful in patients with normal renal function and residual proteinuria
Initial dose: ~ 4 mg/kg/day for CsA and ~ 0.1 mg/kg/day for TAC (reduce in elderly and morbidly obese, increase in children and in malnourished patients)
Slowly taper to the minimal effective dosage once stable remission is achieved
Detailed assessment and frequent monitoring of concomitant medications is paramount, since drug and food interactions with CNI are extensive
Frequently monitor clinical conditions in the first 3 months; any CNI-related side-effect (including serum creatinine increase > 30% from baseline) should prompt dose reduction
CNI blood levels should not be used as the only decision-making tool to adjust doses, as they do not provide adequate information on intracellular drug concentration and pharmacological activity

ineffective intracellular drug concentrations despite paradoxically high blood levels.

Monitoring of T-cell intracellular concentration of CNI and evaluation of P-gp expression on lymphocytes could be potentially useful for predicting the correct CNI dosage, however these methods have not been validated and are not generally available in clinical practice. Moreover, similar CNI intracellular concentrations may not translate to bio-equivalence (i.e. similar immunosuppressive effects) in different patients due to other factors.

Overall, adequate patient selection is the most important action to take when considering CNI treatment for lupus nephritis. Despite some evidence on the short-term efficacy of “multi-target” regimens (CNI + MPA) in selected populations, CNI are currently not indicated in the active phase of SLE, as other drugs such as glucocorticoids, cyclophosphamide, mycophenolate and rituximab may obtain more rapid and consistent effects. For maintenance treatment, patients with pre-existing severe hypertension, diabetes, reduced glomerular filtration rate (≤ 40 mL/min/1.73 m²) or severe tubulo-interstitial lesions at renal biopsy are bad candidates for long-term use of CNI. Ideal candidates to CNI treatment among patients with lupus nephritis would be those with normal renal function and residual proteinuria after induction immunosuppression; this intriguing possibility seems to be confirmed by the data of Argolini et al. [3] but needs to be confirmed by ad hoc prospective clinical trials. The initial dose should be approximately 4 mg/kg/day for CsA and 0.1 mg/kg/day for TAC, but appropriate adjustments would need to be made in case of obesity/malnourishment, very young/old age, subnormal renal function or known drug interactions. The initial dose should be maintained for one month, with slow tapering to the lowest effective maintenance dose [8]. During treatment, the prescription of drugs or the consumption of foods (e.g. grapefruit juice) that may interfere with the activity of cytochrome CYP450 or P-gp should be limited.

CNI doses should be reduced in case of renal (serum creatinine increase of > 25–30% from baseline) or extra-renal toxicity. Evidence of deteriorating renal function

despite CNI reduction may represent a challenge, especially in the absence of other signs of SLE activity. In such cases, it can be difficult to recognize whether the renal injury is caused by CNI nephrotoxicity or by an ongoing activity of lupus nephritis. As pointed out above, CNI blood levels are not reliable, but urinary sediment findings, proteinuria, serum complement, anti-DNA and anti-C1q antibodies may assist the clinician. In difficult cases, kidney biopsy still represents the most valuable tool to reach the correct diagnosis.

In summary, correct CNI handling requires adherence to a few recommendations (Table 1) [8]. Patients should be carefully monitored during the first months of treatment to find the optimal individual dosage, and the prescription of other drugs should take into consideration their possible interaction with CNI. Efficacy and side effects are paramount when evaluating possible dose adjustments, whereas CNI blood levels should not be used as the only decision-making tool. Finally, CNI doses should be gradually tapered to reach the minimal effective dose, which may be significantly lower in case of concomitant use of other immunosuppressive drugs. Applying these simple guidelines to the CNI-based maintenance treatment of lupus nephritis could significantly improve long-term outcomes as shown by Argolini et al., leading to safe and effective control of flares and renal function deterioration even in patients with significant residual proteinuria [3].

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