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Calcineurin Inhibitors: 40 Years Later, Can’t Live Without . . .

Jamil R. Azzi,* Mohamed H. Sayegh,*† and Samir G. Mallat‡

Calcineurin inhibitors (CNIs) revolutionized the field of organ transplantation and remain the standard of care 40 years after the discovery of cyclosporine. The early impressive results of cyclosporine in kidney transplant recipients led to its subsequent use in other organ transplant recipients and for treatment of a variety of autoimmune diseases as well. In this review, we examine the discovery of CNIs, their mechanism of action, preclinical and clinical studies with CNIs, and the usage of CNIs in nontransplant recipients. We review the mechanisms of renal toxicity associated with CNIs and the recent efforts to avoid or reduce usage of these drugs. Although minimization strategies are possible, safe, and of potential long-term benefit, complete avoidance of CNIs has proven to be more challenging than initially thought. The Journal of Immunology, 2013, 191: 5785–5791.

Discovery of calcineurin inhibitors

The introduction of cyclosporine in the early 1980s was a breakthrough in modern medicine, achieved through the multidisciplinary efforts of scientists at Sandoz, now known as Novartis (1). Its addition to the repertoire of immunosuppressive drugs led to a dramatic improvement in the outcome of organ transplant recipients and to its continuous use more than 30 years after its discovery (2, 3).

An immunology laboratory led by Dr. Sandor Lazary, and later by Dr. Jean-François Borel, at the Sandoz company in Basel was established to identify an immunosuppressive agent without major cytotoxicity. For this purpose, a mouse model was developed by Dr. Hartmann Stahelin and Dr. Lazary in which a hemagglutinin test measured the immunosuppressive activity of the administered compound and tumor growth measured its cytotoxic activity (4).

Although cyclosporine was originally derived from the filamentous fungus *Tolypocladium inflatum* Gams in the antibiotic screening program at Sandoz, a sample was sent to Stahelin’s laboratory to test for immunosuppressive and cytostatic activity, with excellent results (5). Drs. Borel and Stahelin described the effect of cyclosporine on T lymphocytes in a paper published in 1976 (6). They showed that, in contrast with other immunosuppressive and cytostatic drugs of that era, cyclosporine demonstrated weak myelotoxicity, avoiding a major side effect of available immunosuppressants (6).

However, it was the lecture given by Dr. Borel at the spring meeting of the British Society for Immunology in April 1976 that stimulated the interest of many scientists and clinicians. As a result, many groups started investigating cyclosporine in animal models and, shortly after, in humans (4).

Close to 10 y later, in the mid-1980s, scientists at Fujisawa Pharmaceuticals isolated another molecule from a soil fungus named *Streptomyces tsukubaensis*, which was given a code FK506 and later named tacrolimus. Cyclosporine and tacrolimus share the same pharmacodynamic property of activated T cell suppression via inhibition of calcineurin (1).

Structure and the mechanism of action of CNIs

Cyclosporine is a cyclic endecapeptide (molecular mass of 1203 kDa) with N-methylated amino acids that make the molecule resistant to inactivation by the gastrointestinal tract and hence usable as an oral immunosuppressive drug (6). Alternatively, tacrolimus is a macrolide antibiotic (molecular mass of 804 kDa). Although it is more soluble in water than cyclosporine, it has a similar high solubility in lipids and other organic solvents (Fig. 1) (1).

CNIs bind intracellular proteins called immunophilins: cyclophilins in the case of cyclosporine A (CsA), and the FK-binding proteins in the case of tacrolimus (also known as FK506). This complex then binds to an intracellular molecule called calcineurin, leading to an inhibition of its activity, and hence inhibiting T cell activation (7). Calcineurin is formed by two subunits: A, which is a catalytic subunit (CnA) responsible for its phosphatase activity, and B, a regulatory subunit (CnB) that is particularly responsive to intracellular calcium and regulates CnA activation (8–11).

T cell activation through TCR stimulation elevates intracellular calcium concentration and activates CnB, which unleashes the phosphatase activity of CnA. Activated CnA dephosphorylates cytoplasmic NFATc, a transcription factor, which causes its translocation, along with the activated calcineurin, into the nucleus where it upregulates the expression of multiple cytokines and costimulatory molecules necessary for full activation of T cells. Among NFAT family members, NFAT1, NFAT2, and NFAT4 are involved in the tran-
scriptional activation of genes encoding cytokines, including IL-2 and IL-4, and CD40 ligand (12). Production of IL-2, in particular, stimulates the growth and differentiation of T cells (13). The cyclophilin/CaA and FK-binding protein/FK complexes directly bind to CaA and inhibit its phosphatase activity. Although inhibiting calcineurin in T cells was shown to suppress T cell activation, more recent data suggest a negative effect of CNIs on regulatory T cell proliferation and function (14, 15). Regulatory T cells have been shown to be essential for immune tolerance induction in transplantation (16). Whether the use of CNIs will be deleterious to any potential tolerogenic therapeutic strategy is still unknown.

Preclinical and clinical studies

As mentioned above, the in vivo T lymphocyte activity of cyclosporine was first described in a paper by Borel and colleagues in 1976. Interestingly, the animal models they used were skin and bone marrow transplants, in addition to an arthritis model (6). Although one of the first challenges that faced the team was the poor water solubility of the molecule, the commitment of Borel and Stahelin to the program motivated them to participate as volunteers in a comparative study to test various galenical preparations for the bioavailability of cyclosporine (4). The method to measure cyclosporine blood levels was developed at the same time (17).

After Borel’s lecture at the British Society for Immunology in London in April 1976, multiple investigators tested cyclosporine in transplant animal models—heart transplant in rat and pig models and kidney transplant in rabbit and dog models (4, 18–20)—with excellent results. These encouraging results in animal studies were translated into human trials around the same time. Seven patients received kidney transplants from cadaveric donors and were treated with cyclosporine as the sole immunosuppressive agent. This short-term study showed that only one patient lost his transplanted kidney due to pyelonephritis, whereas one patient died secondary to disseminated aspergillosis, and five patients were discharged home with functioning grafts between days 22 and 78 after transplant (21). In another study, five patients with acute leukemia who developed graft-versus-host disease following a bone marrow transplant were treated with cyclosporine. The acute erythematous skin reaction of the graft-versus-host disease resolved within 2 d in all the patients; however, four of the five patients died of multifactorial liver failure (22).

The need for new therapeutics in the field of transplantation, in addition to fewer regulations for clinical trials in that era, led to the rapid inclusion of more patients in Calne’s initial pilot study, despite the short term follow-up. One year later, Calne and colleagues published a follow-up paper in The Lancet that included 34 transplant patients treated with CsA. Overall, patients received 32 kidneys, two pancreases, and two livers. Although six patients died of infections, all of them had received additional therapy, five with cyclophosphamide and one with additional steroids. All of the other organs in surviving patients were functioning, and this continued in three patients for more than a year of follow-up (23). Furthermore, in 1981, Starzl et al. (24) showed that 10 of the 12 liver transplant patients treated with cyclosporine and prednisone survived up to 14.5 mo after transplant. However, the nephrotoxicity associated with this new drug quickly became evident; this is discussed in detail below.

These early successes were followed by several multicenter trials. In the European multicenter trial, cyclosporine alone was compared with conventional treatment with azathioprine and prednisolone in 232 recipients of cadaveric renal allografts. The 1 y graft survival was higher with cyclosporine alone compared with azathioprine and steroids (72 versus 52%, respectively; \( p = \)
However, renal function was poorer in the cyclosporine group compared with the conventional therapy group due to the nephrotoxicity of the drug (25). Simultaneously, a Canadian multicenter trial randomized 285 transplant patients to receive either treatment with cyclosporin and prednisone or standard therapy with azathioprine and prednisone. The outcome was strikingly different and in favor of cyclosporin with 1 y graft survival of 84% compared with 67% in the conventional group (26). These trials led the U.S. Food and Drug Administration to approve the use of cyclosporine for prevention of transplant rejection in November 1983, 13 y after its discovery.

Later, the European Collaborative study reported on results from 200 transplant centers that used cyclosporin that showed the superiority of a cyclosporine-based regimen in graft and patient survival (27).

These early studies made cyclosporine a standard of treatment for years to come, opening a new era not only in kidney transplantation but also in other solid organ transplantation. The introduction of cyclosporine improved first-year heart transplant survival to 74% (28). Additionally, the University of Toronto reported in 1986 that the first single lung transplant recipient was alive during a year of follow-up (29, 30). Similarly, the ultimate success in liver transplantation has also been credited to cyclosporine (24). These initial results led to the widespread use of CNIs as part of the immunosuppressive protocols in all solid organ transplantation. Furthermore, cyclosporine became the standard therapy to which all subsequent trials with new modalities were to be compared (31).

Nontransplant use of cyclosporine

The outstanding results of cyclosporine in transplant patients led to its subsequent use in a variety of autoimmune diseases (Fig. 2). Herrmann and Mueller used cyclosporine for a heterogeneous group of polyarthropathies, including psoriasis and rheumatoid arthritis (RA), with encouraging results (32). In 1979, Mueller and Herrmann (33) used cyclosporine to treat four patients with severe psoriasis and noted that the psoriatic plaques “almost disappeared” 5 d after starting treatment.

Ellis and colleagues randomized 85 patients with severe psoriasis to receive increasing doses of cyclosporine for 16 wk. The psoriasis improved in a dose-dependent fashion. Whereas complete clearance of psoriasis was observed in 36, 65, and 80% of the patients receiving 3, 5, or 7.5 mg/kg body weight of cyclosporine, respectively, all three regimens were superior to placebo (34). Currently, topical and oral CNIs remain part of the treatment for mild to moderate psoriatic disease and moderate to severe psoriatic disease, respectively. Forre and colleagues randomized 24 patients with RA to receive cyclosporine at 10 mg/kg/d or azathioprine at 2.5–3 mg/kg/d for 26 wk. Patients treated with cyclosporine showed significant improvement in multiple clinical parameters compared with those treated with azathioprine. Markers of inflammation, such as C-reactive protein and erythrocyte sedimentation rate, improved only in cyclosporine-treated patients (35). With the introduction of newer disease-modifying anti-rheumatic drugs, cyclosporine is currently reserved for RA patients without other treatment options.

Furthermore, multiple trials showed the efficacy of cyclosporine for different immune-mediated clinical entities, such as endogenous uveitis, Sjögren’s syndrome, myasthenia gravis, and Crohn’s disease (36). With the exception of generalized myasthenia, cyclosporine use in these different autoimmune diseases has been significantly reduced owing to its nephrotoxicity and the availability of newer disease-modifying anti-rheumatic drugs.

Because many glomerulonephritides were thought to be immune-mediated, cyclosporine was quickly used to treat proteinuria in patients with nephrotic syndromes including minimal change disease, focal segmental glomerulosclerosis, and membranous nephropathies, with variable results (37). The use of cyclosporine in patients with either steroid-dependent or -resistant minimal change disease revealed a response rate of 60 and 20%, respectively (38). Complete or partial remission of steroid-resistant focal segmental glomerulosclerosis was achieved in up to 60% of the cases treated with cyclosporine (39). Not only do patients with steroid-resistant membranous nephropathy respond to cyclosporine, but in one study, 39% re-
mained in remission after 1 y (40). Tacrolimus is an alternative to cyclosporine in primary membranous nephropathy, as studies have shown a high rate of remission with tacrolimus use (41).

Cyclosporine was later tried in different forms of nephritic syndrome, including IgA nephropathy, membranoproliferative glomerulonephritis, and lupus nephritis (42). However, side effects, particularly nephrotoxicity, remain a major challenge.

To limit the systemic toxicity of the drug, topical cyclosporine was suggested early on to treat ocular and skin lesions of autoimmune diseases, with encouraging results in animal models. Although the efficacy was found to be limited in humans with skin diseases, possibly because of an inability to penetrate the stratum corneum (43), current literature supports the efficacy and safety of topical cyclosporine in the treatment of various ocular surface disorders, particularly dry eye syndrome and chronic allergic keratoconjunctivitis (44). Alternatively, topical tacrolimus is approved for use in atop dermatitis cases, but it has also been used off-label for other skin disorders, mostly as an alternative to topical glucocorticoids (45).

Nephrotoxicity of CNIs

The nephrotoxicity of cyclosporine, reported by Calne and colleagues in the early human studies, remains a major concern for the medical community today (21). Much research has been done to uncover the pathophysiology of cyclosporine’s nephrotoxicity; we divide these studies here into either structural or functional abnormalities.

Functional abnormalities can affect the renal microvasculature or the renal tubules. With regard to the renal microvascularity, afferent arteriolar vasoconstriction resulting from cyclosporine treatment was first reported experimentally in rats (36). Many mechanisms were suggested, including an imbalance between vasoconstrictive and vasodilatory mediators (46), an activation of the renin-angiotensin-aldosterone system (47), an increase in the release of endothelin (5), an increase in free radicals (48), and a sympathetic nerve activation in the native kidneys through synapsin effects (49). Lately, more attention has been focused on endothelial dysfunction in the pathogenesis of acute calcineurin toxicity and thrombotic microangiopathy after transplant (7). With regard to the renal tubules, tubular dysfunction includes hyperkalemia due to aldosterone resistance (50) and a decrease in the Na+/K+ ATPase pump (43), hypomagnesemia due to a decreased expression of paracellin 1 in the thick ascending loop of Henle (45), and hyperuricemia due to a decrease in uric acid tubular secretion (4). A reduced expression of the Na+/K+/2Cl− transporter has also been described and may result in nephrocalcinosis, polyuria, and juxtaglomerular hyperplasia (1).

Structural abnormalities are related to the chronic injury associated with CNIs that is seen in almost all cases by year 10 after transplant (51). All anatomical structures of the kidney may be involved: arterial hyalinosis is frequently irreversible owing to prolonged vasoconstriction or the regulation of NFAT and smooth muscle (14). Tubulointerstitial injuries classically described as stripped fibrosis and tubular atrophy are multifactorial in origin, resulting from an increase in free radicals (15), an upregulation of TGF-β (16) leading to epithelial to mesenchymal transition (52), or an activation of the renin-angiotensin-aldosterone system with an increase in aldosterone (53). Other tubular lesions are seen and include isometric tubular vacuolization and inclusion bodies due to an increase in lysosomes and giant mitochondria (54). The main glomerular lesions include global glomerulosclerosis due to secondary ischemia (44) or focal segmental glomerulosclerosis secondary to hyperfiltration injury (3). Aldosterone seems to be playing a central role in CNI toxicity because aldosterone antagonists may prevent the functional or structural renal lesions (3).

Many risk factors play a role in this nephrotoxicity, including the serum concentration of the drug (34). However, there is an individual susceptibility to chronic nephrotoxicity, as chronic histological changes have been seen with low-dose levels (55), and no significant graft dysfunction was noted in patients exposed to high-dose levels (47). A controversial role played by P glycoprotein, an apical membrane protein necessary for CNI secretion, has also been addressed (56). Finally, the roles of exposure to metabolites of CNIs and to local susceptibility to nephrotoxicity have been reviewed previously (56).

Neurotoxicity, which affects the central and peripheral nervous systems, is another major side effect seen with cyclosporine use. Peripheral tremors are common and headaches may be severe and recurrent. Severe additional symptoms may be present shortly after starting cyclosporine and include seizures, encephalopathy, extrapyramidal syndrome, or posterior leukoencephalopathy (57). The latter, described first in 1996, consists of a reversible syndrome of headaches, altered mental status, seizures, and cortical blindness accompanied by multifocal bilateral white matter abnormalities seen on brain magnetic resonance imaging.

Cyclosporine versus tacrolimus

Although tacrolimus and cyclosporine share the same mechanism of action, they have different toxicity profiles, as described in Table I. The former may be less vasoconstrictive and fibrogenic as compared with the latter, but it may be more diabetogenic across all organ transplants (58–60). Although there is no evidence today that tacrolimus is less nephrotoxic than cyclosporine (61), tacrolimus is currently more commonly used, as some trials showed lower risk of acute rejection compared with cyclosporine (62, 63). The ELITE study randomized 1645 renal transplant recipients to receive either a standard dose of cyclosporine, low-dose cyclosporine, low-dose sirolimus, or low-dose tacrolimus in addition to mycophenolate mofetil (MMF) and steroids. Patients in the standard cyclosporine group did not receive daclizumab induction. At 1 y after transplant, the low-dose tacrolimus recipient group had lower rejection rate and higher graft survival compared with the three other groups (47).

<table>
<thead>
<tr>
<th>Side Effects</th>
<th>Cyclosporin</th>
<th>Tacrolimus</th>
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<tbody>
<tr>
<td>Vasoconstriction</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Fibrogenesis</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Lower serum creatinine (30)</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Better graft survival (31)</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Diabetes</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Tremor</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>+ +</td>
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</tr>
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<td>Gingival hyperplasia</td>
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<tr>
<td>Dyslipidemia</td>
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+ +, More pronounced side effects; +, less pronounced side effects; −, no side effects.
At 3 y after transplant, the low-dose tacrolimus arm continued to have the highest graft survival rate and the least acute rejection (64). In contrast, two other retrospective studies of the United States Renal Data System data found that there was either no difference in allograft survival (65) or improved allograft survival with cyclosporine (66). These conflicting data could be explained by multiple variables in those studies, including dosing and various cyclosporine preparations. Switching patients from cyclosporine to tacrolimus or vice versa to avoid specific toxicities is done in clinical practice and appears to be safe.

Conversion, minimization, and avoidance trials (CNIs: can’t live with, can’t live without)

Although CNIs are associated with renal injury, whether acute or chronic, many trials to minimize or even avoid CNIs, particularly cyclosporine, have been conducted (51, 67–74).

A recent meta-analysis compared CNI-sparing regimens, either through complete avoidance or minimization, to the standard of care, which is CNI-containing regimens (74). Although the 17 minimization studies, which included 4131 patients, showed no difference in acute rejection, CNI-minimizing regimens showed a reduction in graft failure (odds ratio, 0.73; 95% confidence interval, 0.34–1.31). However, the avoidance studies, which substituted CNI regimens with mammalian target of rapamycin (mTOR) inhibitors, showed an increase in graft failure in patients treated with mTOR inhibitors (74).

Similarly, the CONVERT trial randomized 830 renal transplant patients to either continue a CNI-based regimen or convert to mTOR inhibitors (75). Patients with a glomerular filtration rate (GFR) <40 at the time of conversion to sirolimus experienced higher rates of pneumonia and death. Patients with a GFR >40 at the time of conversion to sirolimus had better GFR at 12 and 24 mo but significantly worse proteinuria (75).

The efforts to replace CNIs continued with the introduction of belatacept, which was recently approved by the U.S. Food and Drug Administration for use in kidney transplant recipients (76). Multiple studies evaluated the effectiveness of belatacept as a maintenance therapy in kidney transplant recipients compared with cyclosporine (70, 71, 77). The BENEFIT trial randomized 666 patients to one of three groups: more intensive belatacept, less intensive belatacept, and cyclosporine. All patients received basiliximab induction therapy and mycophenolate mofetil/corticosteroid maintenance. At 12 mo, the belatacept-treated groups were not inferior to cyclosporine maintenance with respect to patient/graft survival. Renal function assessed by measured GFR was better in the belatacept group (measured GFR was 15 ml/min higher in the belatacept groups versus cyclosporine). The incidence of acute rejection and posttransplant lymphoproliferative disorder was higher in the belatacept groups. The cardiovascular and metabolic profiles were better in belatacept groups compared with patients receiving cyclosporine (70). The CTOT-10 experience was presented in May 2013 at the American Transplant Congress in Seattle by Dr. Newell (abstract no. 15). Nineteen patients were randomized to one of three arms. Patients in groups 1 and 2 were induced with alemtuzumab and maintained on MMF with either tacrolimus or belatacept, respectively. Group 3 was maintained on MMF; belatacept with tacrolimus was stopped at 3 mo after transplant. Three out of six patients in group 2 developed vascular thrombosis. Group 3 showed higher incidence of severe organ rejections (three of seven patients) shortly after tacrolimus withdrawal. The high incidence of organ rejection in patients receiving belatacept suggested that it may not be sufficiently immunosuppressive. The CTOT consortium is currently exploring alternative protocols.

All of these trials suggest that while minimization of CNIs may be safe and offer a potential long-term benefit, complete avoidance of CNIs, alternatively, is more challenging with significantly high risks of rejection and/or graft loss at this time.

Future prospects

Although efforts to avoid CNIs are ongoing, more data support the critical role of these drugs in preventing transplant rejections. An alternative would be to reinvent the use of these drugs through minimization protocols or through targeted delivery in an attempt to reduce their systemic distribution and hence their side effects.

Iacono et al. (78) conducted a single-center, randomized, double-blind, placebo-controlled trial of inhaled cyclosporine initiated within 6 wk after transplantation and given in addition to systemic immunosuppression. Interestingly, survival and chronic rejection-free survival were improved with the inhaled cyclosporine compared with placebo, without additional risk of nephrotoxicity. Subsequently, 294 lung transplant patients were enrolled in a multicenter phase III clinical trial of inhaled cyclosporine. This trial seems to have failed to reproduce the prior results (79). Despite these conflicting data, inhaled cyclosporine and tacrolimus remain attractive treatment strategies for lung transplant, given its accessibility to inhaled therapeutic agents. It remains to be seen whether newer formulations of inhaled cyclosporine and tacrolimus will be tested again in future clinical trials.

Our group suggested the use of nanotechnology to reduce the side effects of CNIs. We reported an unprecedented strategy for preparing polylactide–CsA nanoparticles (termed CsA-NPs) through CsA-initiated, ring-opening polymerization of lactide followed by nanoprecipitation. The resulting CsA-NPs have sub–100-nm sizes, narrow particle size distributions, and release CsA in a sustained manner without a “burst”-release effect (80). Both free CsA and CsA-NPs displayed comparable suppression of T cell proliferation and production of inflammatory cytokines in various T cell assays in a dose-dependent manner. Our published and unpublished data in animals show the possibility of using polymeric nanoparticles as a promising drug delivery vehicle for treating diseases with improved efficacy and reduced toxicity (80, 81).

Conclusions

Most transplant recipients still rely on CNIs 40 years after their discovery. Although the discovery of new drugs is of paramount importance to reduce the toxicity of current regimens, CNI-based regimens remain the standard of care in organ transplant recipients. The next challenge facing the transplant community is the development of safe and effective regimens that minimize immunosuppression while preserving graft function and optimal survival.

Disclosures

The authors have no financial conflicts of interest.

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