Review

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Calcineurin Inhibitors in Atopic Dermatitis

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Abstract: Atopic dermatitis (AD) is a chronic inflammatory condition which significantly affects quality of life in both patients and their caregivers. Calcineurin inhibitors (CNI) are a class of immunosuppressive drugs predominantly inhibiting T cell mediated immune processes. CNI form complex by binding with immunophilins and in turn inhibit calcineurin and suppress downstream inflammatory pathway. Tacrolimus and pimecrolimus are topical CNI approved for the treatment of mild to severe AD in both children and adults. Application site reactions are frequently seen. They have been issued with black box warning towards increased risk of malignancies but supportive evidence is lacking. Cyclosporine, though highly effective, is used off-label for AD management. It is used for controlling acute flares of AD due to its very rapid onset of action. Nephrotoxicity and hypertension are its serious complications. CNI have not been reported with teratogenicity and may be used conditionally during pregnancy and lactation.

Keywords: atopic dermatitis; calcineurin; NFAT; tacrolimus; pimecrolimus; cyclosporine

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory remitting and relapsing condition with a global prevalence of 13% and 7% in children and adults respectively [1–4]. It is typically seen in infants and remits before adolescence in up to 70% of the patients [5,6]. AD is characterized by intense itching and sleep disturbance and is associated with reduced quality of life in both patients and their caregivers. The pathogenesis of AD involves a complex interplay between genetics, defective skin barrier, immune function dysregulation and environmental factors.

Calcineurin inhibitors (CNI) are a class of immunosuppressive drugs which predominantly inhibit the activation of T cell mediated immune processes. They were initially used for the prevention of solid organ transplant rejection but are currently utilized for treating various inflammatory and autoimmune conditions in dermatology including atopic dermatitis.

2. Classification

CNI are broadly classified into two types based on the routes of administration and are listed in Table 1.

Table 1. Classification of calcineurin inhibitors [7].

Topical CNI	Systemic CNI
Tacrolimus	Cyclosporine (oral and intravenous)
Pimecrolimus	Tacrolimus (oral and intravenous)
Cyclosporine (ophthalmic solution)	Voclosporin

CNI: Calcineurin inhibitors.

3. Mechanism of Action

Calcineurin, a calcium/calmodilin dependent serine/threonine phosphatase plays a key role in the immune pathway involved in activation of T-cell pathway and release of cytokines. The

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interaction between T cell receptor complex and peptide/MHC II complex on the antigen presenting cells causes release of calcium from the endoplasmic reticulum. Calcineurin gets activated by the elevated intracellular levels of calcium which in turn dephosphorylates a transcription factor, nuclear factor of activated T cells (NFAT). The activated NFAT translocates to the nucleus and initiates transcription of interleukin (IL)-2, the main cytokine required for activation, differentiation and proliferation of helper, suppressor and cytotoxic T cells. In addition, NFAT also mediates the transcription of other pro-inflammatory cytokines such as IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony stimulating factor (GM-CSF) [7,8].

CNI bind with high affinity to cytosolic protein receptors known as immunophilins. There are two immunophilins, namely cyclophilin and FK-binding protein-12 (FKBP12) [9]. Cyclosporine and voclosporin bind to cyclophilin whereas tacrolimus and pimecrolimus bind to FKBP12. The CNI-immunophilin complex binds competitively to calcineurin and inhibits its phosphatase activity resulting in failure of dephosphorylation of NFAT. This prevents nuclear translocation of NFAT leading to suppression of downstream inflammatory cascade of T cell activation.

Various anti- inflammatory actions involving cells other than T lymphocytes are also mediated by the CNI. They inhibit release of histamine from mast cells and basophils, reduce antigen presentation by dendritic cells, decrease substance P release from nerve cells, and increase the expression of defensin and TGF-β and reduce inducible nitric oxide synthase in keratinocytes. An improvement in the epidermal barrier function is also produced by topical CNI [8].

4. Tacrolimus

Tacrolimus is derived from the soil dwelling bacteria *Streptomyces tsukubaensis*. It binds to FKBP12 and the resultant tacrolimus-FKBP12 complex inhibits calcineurin. Tacrolimus is available in both topical (0.03% and 0.1% ointment) and oral formulations but only the topical formulation is approved for the treatment of AD.

The efficacy of tacrolimus for short and long term treatment of pediatric and adult AD patients has been well established. Tacrolimus was found to achieve at least 90% improvement in physician's global evaluation of clinical response in both children and adults as compared to vehicle. There was no significant difference in the pediatric patients treated with either 0.03% or 0.1% ointment while higher concentration was more effective in adults [10,11]. In comparison to placebo, tacrolimus was found to significantly increase the flare-free treatment days as well as duration for first relapse when used prophylactically over a period of 52 weeks [12]. In a meta-analysis which included 25 randomized controlled trials, efficacy and safety of tacrolimus was compared with topical corticosteroids and placebo. Tacrolimus 0.1% was found to be more effective than both hydrocortisone butyrate 0.1% and hydrocortisone acetate 1% while 0.03% was less effective than hydrocortisone butyrate 0.1% but more effective compared to hydrocortisone acetate 1% [13]. The effectiveness of 0.03% and 0.1% ointments is equivalent to medium-low potency (US group 5) and medium potency (US group 4) topical corticosteroids respectively [14].Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

4.1. Indications

Tacrolimus is approved by United States Food and Drug Administration (US FDA) for the short or intermittent long term treatment of moderate to severe atopic dermatitis. The 0.03% ointment is approved for adults and children aged 2 to 15 years whereas 0.1% in patients aged above 15 years. In Europe and Canada, tacrolimus is additionally approved for maintenance therapy to prevent flares and increase the flare-free duration. The manufacturers recommend twice daily application to the affected area whereas the AAAAI/ACAAI Joint Task Force (2023) advices once application per day [14].

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4.2. Adverse Effects

Tacrolimus is generally well tolerated and has an excellent long term safety record. Most commonly observed adverse effects include irritation, burning sensation and itching at the site of application. These are usually mild and transient in nature and subside within few days to few weeks. These tend to be more common when tacrolimus is applied over an acutely inflamed lesion [8]. The burning sensation is hypothesized to be due the release of preformed neurotransmitter, substance P from the nerve endings on applying tacrolimus. Continued application of tacrolimus leads to depletion of substance P and improvement in the symptoms [8,15]. Additional strategies which can be used to reduce the local adverse effects include, pre-cooling the tube in a refrigerator before using, applying emollients prior to tacrolimus, using topical corticosteroids for few days before tacrolimus and co-prescription of non-steroidal anti-inflammatory drug such as paracetamol or ibuprofen during initial few days [14,16]. An elevated but insignificant risk of localized herpes simplex and molluscum infection has been reported occasionally [17]. Other rarely reported adverse effects include acne, dermatitis mimicking rosacea and flushing of the face after alcohol consumption [18–20]. It is recommended to wait till the pre-existing infection subsides before starting topical CNI.

Prolonged usage of topical corticosteroids leads to adverse effects such as atrophy of the skin, striae, and suppression of the hypothalmo-pituitary-adrenal axis. One of the major advantages of tacrolimus is absence of such adverse effects even after using it for a longer period and hence it is preferred for treatment of sensitive areas like face and flexures.

Systemic absorption of topical CNI is associated with the risk of causing immunosuppression, renal toxicity and hypertension. A plasma tacrolimus level of ≥ 5 ng/ml is observed to cause these adverse effects.8 Due to its large molecular size and lipophilic nature, the systemic absorption of topical tacrolimus is negligible. The maximum absorption occurs during the stage of active inflammation and decreases with improvement in the lesion and epidermal barrier restoration.

A black box warning has been issued by the US FDA based on animal studies towards the increased risk of malignancies especially lymphomas associated with topical CNI. No increased risk of malignancy was noted in a long term study involving pediatric AD patients treated with tacrolimus [21]. In a recent meta-analysis which included 110 studies, moderate-certainty evidence was observed regarding the absence of any association towards the development of malignancy with topical CNI [22]. Based on the available evidence, American and European dermatological associations have refuted the elevated risk of malignancy with topical CNI and urged the US FDA to reconsider the warning [23,24].

Patients should be regularly monitored for adverse effects and improvement in the lesions. Treatment should be discontinued if there is no improvement after six weeks. Topical CNI may be considered off-label for treating children younger than 2 years, pregnant and lactating women [25,26].

5. Pimecrolimus

Pimecrolimus is an ascomycin macrolactam developed particularly for the treatment of cutaneous inflammatory conditions such as AD. It is derived from the bacteria *Streptomyces hygroscopicus var. ascomyceticus*. Since the molecular weight and chemical structure of pimecrolimus is almost akin to tacrolimus, both have similar mechanism of action and pharmacokinetics. Pimecrolimus also binds to FKBP12 but has lesser affinity than tacrolimus and the resultant pimecrolimus-FKBP12 complex inhibits calcineurin. Pimecrolimus is available in a 1% cream formulation

In a 24 week study involving adult patients with moderate to severe AD, pimecrolimus was compared with vehicle. Significantly less number of patients in the pimecrolimus group had flares and used topical corticosteroids for rescue therapy. The median time to first flare was also significantly longer in the pimecrolimus group [27]. In another study pimecrolimus was found to be less efficacious compared to both triamcinolone acetonide 0.1% cream and hydrocortisone acetate 1% cream in achieving significant reduction in eczema area severity index (EASI) score [28]. Pimecrolimus was compared with topical tacrolimus in 3 randomized controlled trials and tacrolimus was found to be significantly superior in reducing the EASI score at 6 weeks. The

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superiority of tacrolimus 0.1% was persistent throughout 6 weeks whereas it was only at week 1 for tacrolimus 0.03% [29]. The efficacy of pimecrolimus is between medium-low potency (US group 5) and low/lowest potency (US group 6/7) topical corticosteroids [14].

5.1. Indications

Pimecrlimus is approved by US FDA for the short or intermittent long term treatment of mild to moderate AD in adults and children aged more than 2 years. Similar to tacrolimus, the manufacturer recommends twice daily application while the AAAAI/ACAAI Joint Task Force (2023) advices once application per day [14].

5.2. Adverse Effects

Similar to tacrolimus, pimecrolimus is also associated with transient local adverse effects such as burning, stinging, erythema and itching which subside within few days. Flushing of the face after alcohol consumption, acne, and herpes simplex and molluscum infection have been reported occasionally [30–32]. Pimecrolimus may cause lesser local irritation in the initial days as compared to tacrolimus and may be preferred in patients with milder disease and have higher incidence of skin irritation [29].

A serum pimecrolimus level of ≥15 ng/ml is associated with systemic immunosuppression [33]. Because of its large molecular size and highly lipophilic property, very small quantity gets diffused form the epidermis into the circulation. In a study involving infants who were treated with pimecrolimus, the highest serum concentration detected was 2.26 ng/ml [34]. US FDA has issued black box warning for pimecrolimus also in view of hypothetically increased malignancy risk but supporting evidence is lacking.

6. Cyclosporine

Cyclosporine also known as cyclosporine A (CsA) is a undecapeptide obtained from the soil fungus *Tolypocladium inflatum.* It was initially approved for prevention of solid organ transplant rejection because of its selective T cell mediated immunosuppressive action. It binds to a cytosolic immunophilin called cyclophilin and forms cyclosporine-cyclophilin complex which inhibits calcineurin. CsA does not cause cytotoxicity, bone marrow suppression and teratogenicity which are seen with other immunosuppressants. The immunosuppressive activity of CsA is 50-100 times less than systemic tacrolimus [35].

Being a lipophilic compound, the absorption of orally administerd CsA is poor and shows wide variations. This is particularly a major problem with the original CsA formulation (Sandimmune). A newer hydrophilic microemulsion formulation (Neoral) has been developed to overcome this concern. The microemulsion is associated with better absorption and up to 10-54% higher bioavailability as compared to Sandimmune [36]. The microemulsion is available as capsule (25, 50 and 100 mg) and oral solution (100 mg/5 ml)

Multiple randomized controlled and comparative trials have proven the efficacy of CsA for short and long term treatment of AD in both adults and children. Both low (2.5-3 mg/kg/d) and high (5 mg/kg/d) starting doses of CsA were found to produce similar improvement in EASI and body surface area improvement at the end of two weeks [37]. CsA was found to produce significantly better response than methotrexate, IVIG and phototherapy but the response was comparable with enteric coated mycophenolate sodium [38,39]. Rapid and better response has been observed with higher starting dose as compared to lower dose [38]. On comparing two different regimens of dose reduction, it was observed that starting at higher dose followed by maintaining the same dose but increasing the interval between the doses by 1 day every 2 weeks was equally efficacious as reducing the dose by 1mg/kg/day every 2 weeks [40]. Lowest effective dose is recommended for maintenance therapy [41]. Relapse of AD following withdrawal of CsA has been noted in 50% and 80% patients at 2 and 6 weeks respectively in one study [42] whereas in75% and 86% at 6 and 9 months in other studies [43,44]. In contrast, no evidence for relapse was found in another meta-analysis [45].

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6.1. Indications

Cyclosporine is currently not approved by the US FDA but is used off-label for the treatment of AD. The AAAAI/ACAAI Joint Task Force (2023) conditionally recommends CsA in patients with moderate to severe AD who are refractory, intolerant or unable to use mid to high potency topical treatment and systemic treatment inclusive of biologics [14]. CsA is approved in Europe, Australia and Japan for the treatment of AD in patients aged 16 years and above [46]. European guideline (EuroGuiDerm) on atopic eczema recommends CsA to achieve disease control in AD patients who are candidates for systemic treatment in the following doses [26]:

a) Adults: 2.5–5 mg/kg/day in two divided doses

- Acute flare, short-term: 4–5 mg/kg/day
- Long-term: 2.5–3 mg/kg/day

b) Children: 2.5–5 mg/kg/day in two divided doses

It is recommended to combine CsA with emollients and topical anti-inflammatory agents as per necessity. The maximum duration for continuous therapy with CsA is limited to 1 year in the US and 2 years in Europe [47,48]. The dose should be taken on an empty stomach at the same time everyday to achieve higher serum concentration and to reduce intraindividual variation [49]. The oral solution can be mixed in apple or orange juice or milk to increase its palatability [50]. CsA is administered in two regimens, that is, starting with high dose followed by gradual tapering or initiating with low dose followed by increasing the dose. The regimen to be used depends upon the severity of the disease.

6.2. Pretreatment Assessment and Monitoring

Before starting CsA a baseline evaluation including detailed history, clinical examination and investigations should be undertaken. Careful monitoring is necessary due to the narrow therapeutic index of CsA. Table 2 lists the parameters to be assessed at baseline and during follow up. In high risk patients and in patients with altered laboratory parameters more frequent examination may be necessary.

Table 2. Guidelines of monitoring for cyclosporine therapy [49].

Parameter	Time of evaluation
History and physical examination [Pre-existing illnesses such as hypertension, kidney and liver disease, malignancy and previous or current infections (tuberculosis, hepatitis B and C and herpes simplex)]	Baseline and during every follow up visit
Blood pressure (Two separate measurements <140/90 mm Hg)	Baseline, week 2, 4, 6, and 8, and then every month
Serum creatinine (Mean of two fasting measurements; to repeat if >10% difference)	Baseline, week 2, 4, 6, and 8, and then every month
Blood urea nitrogen	Baseline, week 2, 4, 6, and 8, and then every month
Complete blood count, Liver function tests, Lipid profile, Serum potassium, magnesium and uric acid and Urine analysis	Baseline, monthly
Glomerular filtration rate	After 1 year continuous therapy

6.3. Adverse Effects

CsA is associated with both cutaneous and systemic adverse effects. Nervous system associated effects are most frequent and may be seen with very short term usage. Renal dysfunction and

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hypertension are the most feared complications. The mechanism behind various adverse effects caused by CsA is not clear. Mitochondrial dysfunction and inhibition of immunophilins regulating the mitochondrial ion channels is implicated [51] Most of the side effects are dependent on dose and duration of treatment and reversible on stopping therapy. Generally CsA is tolerated better by children than adults. Various adverse effects caused by cyclosporine are listed in Table 3.

Table 3. Adverse effects associated with cyclosporine [49].

Mucocutaneous	Gingival hyperplasia, hypertrichosis, acneiform eruptions
Renal	Functional impairment – vascular and tubular dysfunction Structural impairment
Cardiovascular	Hypertension
Malignancy	Non-melanoma skin cancers, lymphoma
Neurologic	Headache, tremors, paresthesia, seizures, psychosis, sleep disturbance
Gastrointestinal	Nausea, abdominal pain, diarrhea
Musculoskeletal	Fatigue, lethargy, arthralgia, myalgia
Laboratory abnormalities	Hypertiglyceridemia, hypomagnesemia, hyperuricemia, hyperkalemia, anemia, thrombocytopenia, hyperbilirubinemia, elevated transaminases

Compliance with the current dermatological guidelines has significantly reduced the risk of renal toxicity. Acute renal damage causing functional impairment is usually reversible whereas chronic renal impairment is often irreversible. If there is more than 30% increase in the serum creatinine levels above baseline in two successive readings taken 2 weeks apart, the dose of CsA should at least be reduced by 1 mg/kg /day. CsA should be stopped if creatinine levels are still elevated above 30% from baseline after one month and restarted only if creatinine levels reduce to 10% from baseline [49] CsA induced nephrotoxicity is less common in children and this is attributed to reduced sensitivity and bioavailability or higher clearance of the drug [45,49]. If two consecutive blood pressure readings taken 2 weeks apart are above 140 (systolic) or 90 (diastolic) mm of Hg, either dose of CsA should be reduced by 25-50% or a dihydropyridine calcium channel blocker other than nifedipine (amlodipine or isradipine) should be started [49]. Though an increased risk of malignancy has been reported in transplant patients receiving CsA, majority of these patients received additional immunosuppressant concurrently. In a systematic review assessing the risk of skin malignancies in more than 300,000 renal transplant patients receiving systemic CNI (CsA and tacrolimus), a higher risk of melanoma and non-melanoma skin cancers was observed. However, the risk was not significant as compared to other immunosuppressants [52].

6.4. Contraindications

Severe renal dysfunction, uncontrolled hypertension, severe infections, current or past history of malignancy, radiation therapy and a high cumulative dose of preceding psoralen and ultraviolet A therapy are absolute contraindications for starting CsA. Relative contraindications include severe hepatic derangement, cutaneous infections, seizure disorders, immunodeficiency disorders, premalignant conditions, elderly age and alcohol abuse [49]

6.5. Drug Interactions

CsA is metabolized completely in the liver by cytochrome P450 IIIA enzyme system. Medications which can induce or inhibit this enzyme system should be used cautiously along with cyclosporine. Grapefruit juice should be avoided to mix CsA oral solution as it increases its levels by inhibiting the intestinal cytochrome P450 enzyme [49]. Other nephrotoxic drugs should also be prescribed carefully along with CsA. Tetracyclines are contraindicated along with CsA as they increase the risk of pseudotumor cerebri [53].

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6.6. Special Situations

CsA is known to cross the placental barrier and get excreted in the breast milk. It is classified as 'probably compatible' during pregnancy as per newer FDA ratings. There are no reports of teratogenicity associated with CsA. In a meta-analysis of 15 studies, no significant increase in the incidence of congenital malformations was noted. Although there was a trend towards prematurity and low birth weight, it was not significant [54]. No adverse effects have been reported in breastfed infants whose mothers were on CsA [55,56]. As per European guideline (EuroGuiDerm) on atopic eczema, CsA can be considered in pregnant woman with severe AD and recommend it as the drug of choice where systemic therapy is likely to be required throughout pregnancy [25,26].

7. Conclusions

AD can severely affect the physical, psychological and economical condition of both patients and the caregivers. Topical CNI are effective and safe options for the prolonged treatment of mild to severe AD especially in those who cannot use topical corticosteroids or have steroid phobia. Cyclosporine although unapproved, remains one of the most effective drugs to control the acute flares of AD due to its rapid onset of action. Judicious use of CNI still has a significant role to play in the management of AD in the era of newer biologics and Janus kinase inhibitors.

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