

Mast Cells as Targets of Pimecrolimus

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Abstract: Mast cells, the multi-functional secretory cells, are the pivotal effector cells in immune response, and contribute to the pathogenesis of many diverse diseases, like asthma and mastocytosis, by releasing numerous proinflammatory mediators. Pimecrolimus (SDZ ASM 981) is a derivative of the macrolactam ascomycin and is a member of the calcineurin inhibitor class of immunosuppressors. It inhibits the calcineurin-dependent activation of nuclear factor of activated T cells and the expression of a number of proinflammatory cytokines in turn. Pimecrolimus has high and selective anti-inflammatory activity within the skin, and with much lower potential to affect local and systemic immune responses. Therefore it has been widely used for treatment of various inflammatory skin diseases. It has a cell-selective mode of action, and mast cells are its specific target cells. Pimecrolimus inhibits the release of both preformed and *de novo* synthesized mediators from activated mast cells and inhibits accumulation of mast cells by inducing apoptosis. Several experimental and clinical reports have demonstrated the successful application of pimecrolimus and other calcineurin inhibitors, such as tacrolimus and cyclosporine A, to treat mastocytosis, a spectrum of disorders characterized by mast cell hyperplasia, especially cutaneous mastocytosis. These new findings suggest that pimecrolimus and other calcineurin inhibitors may be a novel and effective therapeutic approach for mast cell-associated diseases such as asthma and mastocytosis.

Keywords: NFAT, macrophilin-12, immunosuppression, calcineurin, cell degranulation, apoptosis, mastocytosis, therapy.

1. INTRODUCTION

Mast cells are multi-functional secretory cells, which are characterized by numerous large cytoplasmic granules. Mast cells are found in almost all major organs and tissues of the body, especially near blood vessels and nerves and in proximity to surfaces that interface the external environment such as respiratory and gastrointestinal system and the skin. Mast cells in adults are derived from CD34⁺ hematopoietic progenitor cells of bone marrow and then migrate into the vascularized tissues. Upon stimulation, mast cells release various mediators, which can be mainly classified into three categories: a) the extracellular release of preformed mediators stored in the cytoplasmic granules, such as histamine, neutral proteases, proteoglycans, and several cytokines and growth factors, by the process of degranulation; b) the synthesis and release of lipid mediators, such as prostaglandins and leukotrienes; and c) the *de novo* synthesis and secretion of cytokines, growth factors and chemokines [1, 2]. Moreover, stimulated mast cells also newly synthesize reactive oxide species, which play their role in regulation of functions of mast cells and mast cell-dependent inflammatory response [3]. The mediators released from mast cells not only directly contribute to the immune responses but also regulate the functions of other proinflammatory cells in the paracrine manner; their targets include T cells, eosinophils and dendritic cells. Mast cells play an important role in tissue homeostasis, wound healing, tissue remodeling, immediate hypersensitivity and inflammatory reactions, and host defense [1, 2]. Abnormal activation and hyperproliferation of mast cells contribute to the pathogenesis of many diverse diseases such as asthma and mastocytosis by releasing numerous proinflammatory mediators [4, 5].

Pimecrolimus (SDZ ASM 981, Elidel[®]), a lipophilic molecule with a molecular weight of 810 Da, is the 33-epichloro-derivative of the macrolactam ascomycin (Fig. 1). Ascomycin was first isolated as the fermentation product of *Streptomyces hygroscopicus* var. *ascomycetus* in early 1960's, and its immunosuppressive prop-

erties were discovered in the 1980's [6]. Pimecrolimus was synthesized during the development process of this class of drugs [6]. Ascomycin macrolactams are immunophilin ligands that bind to a specific cytosolic receptor. Pimecrolimus binds with high affinity to macrophilin-12, which is also the intracellular receptor of tacrolimus (FK506) and known as FK506 binding protein. Like other calcineurin inhibitors, such as tacrolimus and cyclosporine A (CsA) (Fig. 1), binding of pimecrolimus with macrophilin-12 forms an inhibitory complex and then binds to calcineurin. Calcineurin is a cytoplasmic calmodulin-dependent serine/threonine protein phosphatase that is present in many types of cells, including lymphocytes, dendritic cells and mast cells. It is activated by the elevation of intracellular Ca²⁺ and is implicated in various Ca²⁺-dependent signal transduction pathways. The consequence of calcineurin activation is to dephosphorylate its cytosolic substrates, including nuclear factor of activated T cells (NFAT) [7]. NFAT is an important transcription factor responsible for transcription of a number of growth factors and inflammatory factors. NFAT molecules are heavily phosphorylated and reside in the cytoplasm as inactive form in resting cells. After dephosphorylation by activated calcineurin, they translocate from cytoplasm to the nucleus and in turn activate gene transcription of many cytokines, including interleukin (IL)-2, IL-3, IL-4, tumor necrosis factor- α (TNF- α), interferon- γ , transforming growth factor- β , and granulocyte-macrophage colony-stimulatory factor [8]. Pimecrolimus inhibits this pathway through binding to macrophilin-12 to form inhibitory complexes that block the phosphatase activity of calcineurin and nuclear translocation of NFAT in turn, and then results in the suppression of the generation of proinflammatory cytokines. Other calcineurin inhibitor class of immunosuppressants, such as CsA and tacrolimus, suppress the immune response through the similar mechanism but CsA binding to different type of cytosolic immunophilin, cyclophilin [9].

Pimecrolimus has a cell-selective mode of action, and its specific target cells include T cells and mast cells [6, 10]. Pimecrolimus inhibits T cell proliferation, activation and release of a number of growth factors and pro-inflammatory mediators at sub-nanomolar concentrations *in vitro*, especially T-helper type-1 cell (T_H1) and T_H2 type cytokines [6, 10]. Topical pimecrolimus appears not to alter the morphology, phenotype, and the numbers of Langerhans cells (e.g., resident dendritic cells) in skins, whereas the

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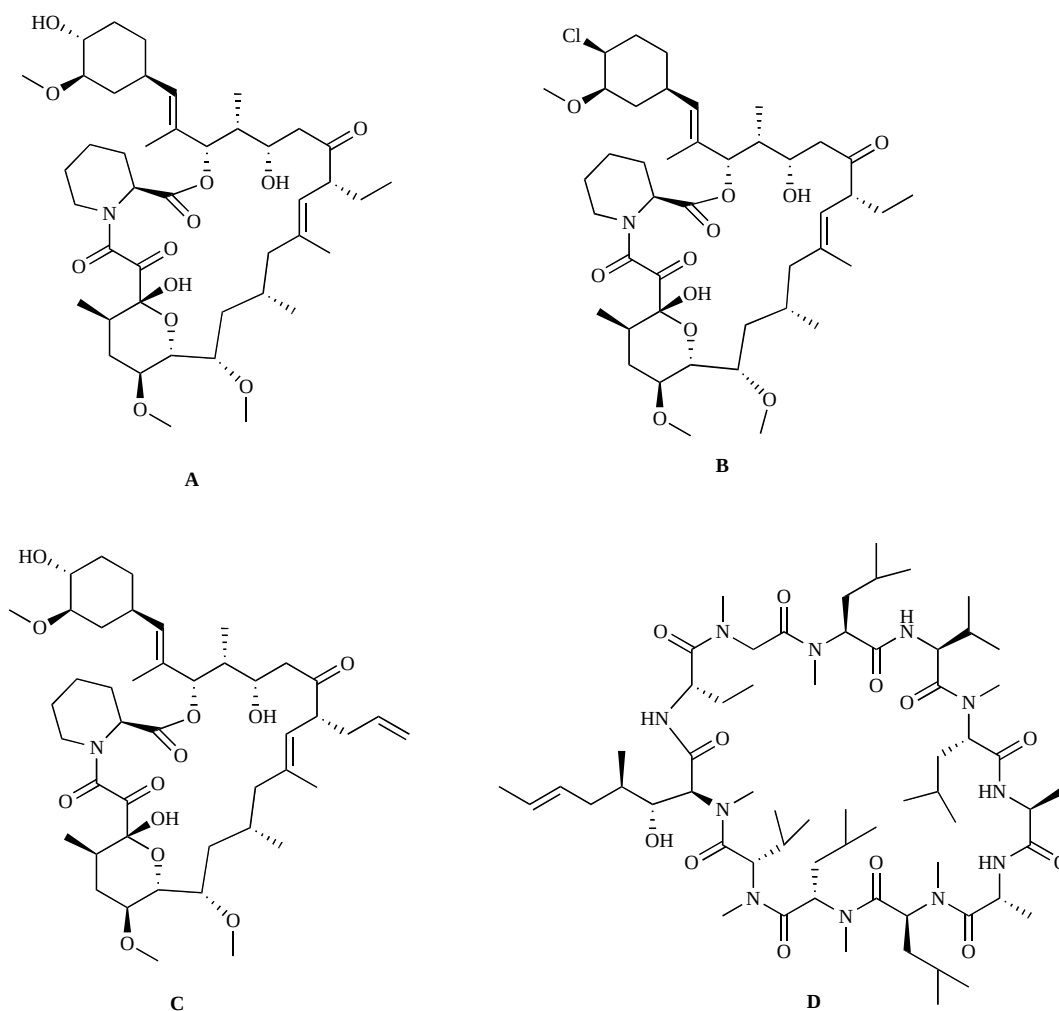


Fig. (1). Chemical structures of (A) ascomycin; (B) pimecrolimus; (C) tacrolimus; and (D) cyclosporine A.

numbers of inflammatory DCs (e.g., inflammatory dendritic epidermal cells and plasmacytoid dendritic cells) are reduced [11-14]. With numerous animal experiments and clinical studies, pimecrolimus demonstrates the favorable pharmacological profile due to its highly lipophilic nature [6, 10]. Compared to corticosteroids and tacrolimus, topical pimecrolimus has high and selective anti-inflammatory activity within skins, minimal percutaneous absorption and much lower potential to affect local and systemic immune responses. Furthermore, even with systemic administration pimecrolimus also shows a lower potency for systemic immunosuppression compared to tacrolimus, although they bind to the same intracellular receptor, macrophilin-12. Pimecrolimus, thus, combines high anti-inflammatory activity in skin with a low potential to impair systemic immune reactions, which make it has been a prevalent alternative to topical corticosteroids for the treatment of a number of inflammatory skin diseases, including atopic dermatitis, allergic contact dermatitis, irritant contact dermatitis, psoriasis, lupus erythematosus and vitiligo [15-17].

In this review, we summarize the current knowledge on the effects of pimecrolimus on mast cells and associated molecular mechanisms.

2. PIMECROLIMUS INHIBITS RELEASE OF MEDIATORS FROM ACTIVATED MAST CELLS

The activation of mast cells results in secretion of preformed mediators in their granules and the synthesis and secretion of lipid

mediators and cytokines, and these released mediators carry out the effector functions of mast cells in immune response. Mast cells are one of the well-demonstrated specific targets of pimecrolimus. The inhibition of release of mediators from activated mast cells is the firstly identified activity of pimecrolimus to mast cells [6, 10]. Pimecrolimus inhibited exocytosis of preformed mediators (e.g. serotonin) with an IC_{50} of ~30 nM, and transcription and release of newly synthesized mediators (e.g. $TNF-\alpha$) with an IC_{50} of 100 nM in the rat basophilic leukemia (RBL)-2H3 cell line stimulated by high affinity IgE receptor ($Fc\epsilon RI$)-mediated activation, and the mechanism of action involved the formation of calcineurin inhibitory complexes with macrophilin [18]. Pimecrolimus also inhibited the secretion of preformed mediator hexosaminidase from the murine mast cell line C117 activated by $Fc\epsilon RI$ crosslinking with an IC_{50} of 26.7 nM. Meanwhile, it inhibited the cytokine release, such as IL-2, 4, 10 and interferon- γ , from stimulated T-cell clone CFTS4:3.1 cells with an IC_{50} of 0.2 to 0.42 nM and inhibited the proliferation of T cell stimulated via monocyte-derived dendritic cells, B cells or $\alpha CD3$ with an IC_{50} of 0.78 to 2.6 nM [19]. Preincubation of primary cultured human dermal mast cells with pimecrolimus showed a dose-dependent inhibition of anti-IgE-induced histamine release, whereas the spontaneous histamine release was not affected. The inhibitory capacity of pimecrolimus exceeded that of CsA and dexamethasone. The anti-IgE-mediated release of tryptase was inhibited similarly with histamine by pimecrolimus. Although the effects were less pronounced, the *de novo* generation of

leukotriene C4 was also inhibited. As with histamine and tryptase, TNF- α release from calcium ionophore/phorbol myristate acetate-stimulated dermal mast cells was strongly inhibited by pimecrolimus [20]. Moreover, as in mast cells, pimecrolimus also caused a strong inhibition of anti-IgE-induced histamine release from human basophils in a dose-dependent manner, while the spontaneous histamine release is not affected [20]. Other calcineurin inhibitors, such as CsA and tacrolimus, were also found to inhibit the release of preformed and *de novo* synthesized mediators from mast cells provoked by either stem cell factor, Fc ϵ RI cross-linking or calcium ionophore [18-22].

NFAT is one of the major transcription factors activated by calcineurin in stimulated mast cells, which is responsible for the production of various cytokines and growth factors [23]. As noted earlier, pimecrolimus, a calcineurin inhibitor, inhibits the activation of NFAT, and in turn the NFAT-dependent transcription of cytokines. This can interpret the pimecrolimus-mediated inhibition of the synthesis and release of some cytokines (e.g. TNF- α). However, the mechanisms for pimecrolimus inhibiting the release of the preformed mediators (such as histamine) have not been demonstrated elaborately. Phosphatases are involved in IgE-dependent mast cell exocytosis [24]. Recently calcineurin was found being involved in regulating degranulation of mast cells. With the utility of a siRNA knockdown functional screen, calcineurin B, the regulatory subunit of calcineurin, was identified to play important role in regulating Fc ϵ RI-induced degranulation in murine mast cell line MMC-1. In the calcineurin-B-deficient cells, the protein kinase C activity and IgE/antigen-induced Erk and p38 phosphorylation were reduced, which may be implicated in calcineurin B knockdown-mediated inhibition of mast cell degranulation [25]. Calcineurin may regulate mast cell degranulation through affecting mitochondrial dynamics. In IgE/antigen-stimulated human umbilical cord blood-derived mast cells or substance P-stimulated human mast cell line LAD2 cells, the secretion of preformed TNF was accompanied with mitochondrial translocation from a perinuclear location to exocytosis sites and elevated calcineurin activity, and these processes were calcium-dependent. Dynamin-related protein 1 (Drp1) is one of the most important regulators of both mitochondrial fission and translocation. Block of mitochondrial translocation with a Drp1 inhibitor mdivi-1 or siRNA for Drp1 caused significant inhibition of mast cell degranulation [26]. Drp1 is in the cytoplasm in an inactive form in resting cells, and dephosphorylation at Ser-637 by calcineurin is critical to recruit Drp1 from the cytoplasm to the mitochondrial outer membrane [27]. These results suggest that calcineurin may regulate mast cell degranulation *via* regulating mitochondrial translocation through controlling Drp1 dephosphorylation. Pimecrolimus may repress mast cell degranulation by inhibiting above calcineurin-mediated activities, which needs to be studied further. Moreover, Narenjkar *et al.* [22] demonstrated in rat RBL-2H3 mast cell line that both CsA and tacrolimus inhibited anti-IgE-induced histamine and hexosaminidase release. They further found that such inhibitory activity was associated with two interrelated mechanisms: interfering with membrane repolarization phase, which is essential for optimal mediator secretion, and reducing increase in intracellular free calcium concentration probably *via* inhibiting Ca²⁺ release from intracellular stores. Whether pimecrolimus inhibits the preformed mediators release by mast cells through the similar mechanisms needs to be verified in future.

Furthermore, *in vivo* experiments showed that topical application of pimecrolimus induced skin mast cell degranulation transiently through an indirect mechanism [28]. Balb/c mice were treated with 1% pimecrolimus cream or 0.1% tacrolimus ointment, and the release of substance P and calcitonin generated peptide from primary afferent nerve fibers in mouse skin was observed. The released neuropeptides bound to mast cells, and led to their degranulation. The released mast cell mediators, such as histamine and tryptase, may cause the initial side effects during therapy with

pimecrolimus or tacrolimus, such as pruritus and burning. These results also suggest that the interactions of pimecrolimus and mast cells *in vivo* are complicated.

3. PIMECROLIMUS CAUSES MAST CELLS DEPLETION BY INDUCTION OF APOPTOSIS

In atopic dermatitis patients receiving topical pimecrolimus, the numbers of skin-infiltrating T cells decreased significantly by the induction of apoptosis [13]. Recently, pimecrolimus was also shown to induce apoptosis of mast cells *in vivo* in a murine cutaneous mastocytosis model [29]. The murine model of cutaneous mastocytosis was induced in Balb/c mice by subcutaneous injection of recombinant murine stem cell factor (rmSCF), the principal human mast cell growth factor, and pimecrolimus 1% cream was applied topically and daily. With pimecrolimus treatment, the severity of cutaneous mastocytosis was significantly reduced accompanied with sharply decreased cutaneous mast cell numbers compared with the mice receiving SCF alone, whereas the numbers of neutrophils were not affected. The possible mechanisms for the increased density of cutaneous mast cells caused by SCF administration may include stimulating proliferation of mast cells or progenitor cells resident in the skin or immigrating from the blood, enhancing immigration of mast cell progenitors, or reducing apoptosis of mast cells, and pimecrolimus may inhibit SCF-induced accumulation of mast cells through disturbing one or more of these processes. Pimecrolimus was further shown to cause extensive apoptosis of cutaneous mast cells, which was demonstrated with *in situ* terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay [29]. These results suggest that pimecrolimus is capable to inhibit SCF-induced accumulation of cutaneous mast cells through the induction of apoptosis, which contributes to the improvement of cutaneous mastocytosis symptoms.

The mechanisms for pimecrolimus-induced mast cell depletion and apoptosis *in vivo* remain elusive. It has been reported that pimecrolimus caused an almost complete disappearance of skin plasmacytoid dendritic cells in patients with atopic dermatitis; however it did not alter the viability of cultured plasmacytoid dendritic cells *in vitro*, suggesting the indirect mechanisms were involved *in vivo* [14]. As pimecrolimus-mediated *in vivo* mast cells depletion and apoptosis, this drug may induce mast cells apoptosis either through the direct apoptosis-inducing activity or through indirect mechanisms as it did to plasmacytoid dendritic cells. There have been several reports to study the effects and mechanisms of other calcineurin inhibitors, such as tacrolimus and CsA, on the proliferation and survival of mast cells and their CD34⁺ progenitors *in vitro*, which may help to understand the effects and mechanisms of pimecrolimus-mediated mast cells depletion and apoptosis.

Tacrolimus and CsA were found to inhibit directly the SCF dependent proliferation and survival of cultured mast cells through the *in vitro* induction of apoptosis [30]. SCF binds to its receptor, c-Kit, and stimulates the differentiation and proliferation of mast cells from CD34⁺ cells. SCF also promotes chemotaxis, adhesion and activation of mast cells. Furthermore, it enhances the survival of mast cells by inhibiting apoptosis [31]. The survival and proliferation of murine mast cell line MC/9 were dependent on both IL-3 and SCF. The addition of SCF in the absence of IL-3 maintained MC/9 cell survival but no cell proliferation was detected. The combined addition of IL-3 and SCF to the culture medium resulted in a stronger MC/9 cell proliferation than the addition of IL-3 alone. Tacrolimus and CsA were found to inhibit the SCF dependent, but not the IL-3 dependent stimulatory effects on MC/9 cell proliferation/survival through inducing apoptosis directly. However, SCF-mediated signal transduction in MC/9 cells, including c-Kit tyrosine phosphorylation and MAP kinase nuclear translocation, were not affected by tacrolimus or CsA treatment [30]. Concluded, calcineurin inhibitors can selectively inhibit SCF-dependent mast cell proliferation/survival and induce mast cell apoptosis, but only some

of the SCF-mediated signaling pathways were affected by these drugs.

FcεRI-dependent stimulation not only causes the release of various mediators and gene expression but also enhances mast cells survival, and the latter process is mediated by the upregulated anti-apoptotic Bcl-2 family member *A1/Bfl-1* gene. Ullerås *et al.* [32] reported that FcεRI-mediated activation induced A1 gene expression, and it was regulated by the calcineurin/NFAT pathway. CsA was found to reduce FcεRI-mediated mast cells survival through inhibition of calcineurin/NFAT pathway and subsequent reduced A1 protein expression. The calcineurin/NFAT pathway also plays important roles in regulating proliferation and differentiation of CD34⁺ cells, which include also the mast cell progenitors. The *in vitro* proliferation of CD34⁺ cells cultured in the presence of FMS-like tyrosine kinase 3 ligand, SCF, granulocyte macrophage-colony stimulating factor (GM-CSF), IL-3, and G-CSF was profoundly inhibited by treatment with CsA in a dose-dependent manner [33]. This means calcineurin inhibitors may reduce SCF-mediated mast cell accumulation by the inhibition of proliferation of their progenitors.

Another possible indirect mechanism for pimecrolimus-induced mast cells depletion and apoptosis may be associated with the growth factor withdrawal effects due to T cell apoptosis induced by pimecrolimus [13]. The accurate and detailed effects and mechanisms of pimecrolimus-mediated mast cell depletion and apoptosis, however, needed to be further verified both *in vivo* and *in vitro*.

The calcineurin/NFAT pathway has recently been implicated in regulating cell cycle, survival, proliferation and apoptosis. It plays an important role in regulation of proliferation of a number of types of leukemia and solid tumors. Inhibition of this pathway leads to reduce them proliferation and apoptosis, which may present a novel therapeutic target for human malignancies [34]. In NFATc3-deficient mice, the rate of apoptotic thymocytes was significantly increased [35]. The calcineurin/NFAT pathway directly regulates the expression of a number of genes associated to cell cycle control and proliferation, and its important roles in regulating cell cycle progression, especially G1/S checkpoint, have been well verified. Interference of this pathway with calcineurin inhibitors was found to inhibit proliferation and induce apoptosis in a number of types of cells. In a human pancreatic cancer cell line Panc-1, inhibition of calcineurin/NFAT pathway with CsA or siRNA-mediated knock-down of NFATc1 led to the block of the cell cycle progression and anchorage-independent cell proliferation [36]. In the cell lines derived from B cell lymphoma and T cell acute lymphoblastic leukemia the NFAT activation was increased, which was inhibited by CsA and tacrolimus, and CsA treatment inhibited the cell cycle progression and induced apoptosis of these cell lines. CsA or tacrolimus treatment also inhibited leukemia regression by reduced tumor cell proliferation *via* induction of apoptosis in the mouse xenotransplant models of human T-cell lymphoma *in vivo* [37, 38]. The proliferation of pancreatic β-cells was also inhibited by tacrolimus through induction of apoptosis. Further studies show that PI3K/Akt pathway and D-cyclins expression, the downstream targets of calcineurin, were repressed [39]. The activities of D-cyclin/cyclin-dependent kinase (Cdk) complexes are critical to cell cycle progression from G₁ to S phase and cell proliferation, and their expression is directly regulated by the calcineurin/NFAT pathway [40]. Recently calcineurin/NFAT pathway was also found to be involved in TNF-α-induced apoptosis of human neuroblastoma cell line SK-N-MC [41]. These results suggest that theoretically, pimecrolimus, the selective calcineurin inhibitor, may be capable to inhibit mast cell survival/proliferation and induce mast cell apoptosis through inhibiting the calcineurin/NFAT pathway.

Calcineurin also showed apoptotic activity in some studies, in which it induced Ca²⁺-triggered apoptosis of mammalian cells in the absence of growth factors [42]. The mechanism for calcineurin-

induced apoptosis is *via* dephosphorylation of BAD, a pro-apoptotic factor and member of the Bcl-2 family. Upon activation, calcineurin dephosphorylated BAD, which subsequently translocated from cytosol to mitochondria. BAD bound Bcl-X2 to form the heterodimer in mitochondria and then triggered apoptosis. Calcineurin inhibitors inhibited the calcineurin-induced BAD translocation and apoptosis [43]. In a vulvar lichen sclerosus patient after two months of topical pimecrolimus treatment, the number of apoptotic p53 immunopositive keratinocytes decreased, and anti-apoptotic Bcl-2 expression was increased, which suggests that pimecrolimus appears to repress but not induce apoptosis in keratinocytes [44]. These results suggest that the roles of the calcineurin/NFAT pathway for apoptosis are cell type specific, and the effects of pimecrolimus on cell proliferation and apoptosis vary in different types of cells or under diverse pathophysiological status.

4. APPLICATION OF PIMECROLIMUS AND OTHER TOPICAL CALCINEURIN INHIBITORS IN TREATMENT OF MASTOCYTOSIS

Mastocytosis represents a spectrum of clinical disorders characterized by mast cell hyperplasia in various organs [5, 45]. The skin is the organ most commonly affected by mastocytosis, which is termed cutaneous mastocytosis. Other types of mastocytosis include systemic mastocytosis, mast cell sarcoma, and extracutaneous mastocytoma (Table 1) [46]. The excessively accumulated mast cells secrete multiple mediators that cause the clinical symptoms of cutaneous mastocytosis. The current treatment of cutaneous mastocytosis is mainly symptomatic and directed towards the skin lesions and systemic signs caused by the release of mast cell mediators [47, 48]. Pimecrolimus and other topical calcineurin inhibitors (such as tacrolimus) not only inhibit the release of these mediators but also repress mast cell proliferation by inducing apoptosis. When combined with their favorable pharmacological profile, the available data suggest that this class of drugs may be the novel effective therapeutic candidates for cutaneous mastocytosis, and possibly also for other types of mastocytosis.

Table 1. Classification of Mastocytosis

Cutaneous mastocytosis
☐ Urticaria pigmentosa
— Typical — Plaque form — Nodular form — Telangiectasia macularis eruptiva perstans
☐ Diffuse cutaneous mastocytosis
☐ Mastocytoma of skin
Systemic mastocytosis (SM)
☐ Indolent SM
☐ SM with an associated clonal hematologic non-mast cell lineage disease
☐ Aggressive SM
☐ Mast cell leukemia
Mast cell sarcoma
Extracutaneous mastocytoma

So far, there are only very limited data on the use of pimecrolimus in treatment of cutaneous mastocytosis. Telangiectasia macularis eruptiva perstans (TMEP) is a rare form of cutaneous mastocytosis that occurs exclusively in adults. One TMEP patient was successfully treated with topical application of pimecrolimus [49]. Recently, Correia *et al.* [50] reported two pediatric cutaneous mastocytosis cases treated with topical pimecrolimus. Two patients, a 14-month-old girl and a 26-month boy, with localized cutaneous erythematous lesions with positive Darier sign, a prevalent clinical sign for pediatric cutaneous mastocytosis, were treated with pimecrolimus cream twice daily combined with oral antihistamine loratadine. The dense mast cell infiltrate with perivascular and interstitial location was observed with the punch skin biopsy in patients at diagnosis. In both patients the almost complete response was achieved after four months of treatment, and no recurrence was observed after four years and two years follow-up, respectively. However, the changes of mast cells numbers before and after treatment were not evaluated in these studies. They also treated four more children with urticaria pigmentosa using pimecrolimus cream and oral antihistamine and significant improvement was observed three months after treatment [50]. In a murine model of cutaneous mastocytosis, topic application of pimecrolimus 1% cream daily for ten days significantly reduced the clinical signs of cutaneous mastocytosis, including erythema, wheal after rubbing lesion skins and increased thickness of skin, and the numbers of cutaneous mast cells and the levels of skin histamine were reduced after treatment [29]. The inhibitory effect of pimecrolimus on skin histamine levels appears to be due to its combined suppressive effects on both mast cell accumulation and mast cells degranulation. These combined clinical and experimental studies suggest that pimecrolimus may be an effective candidate to treat cutaneous mastocytosis.

There have been also several reports to apply other calcineurin inhibitors, such as tacrolimus and CsA, to treat mastocytosis. Fujino *et al.* [51] first reported the tacrolimus-mediated inhibition of intestinal mastocytosis induced by the infection of trematode *Echinostoma trivolvis* (Platyhelminthes: Trematoda) in C3H mouse strain. Repressed intestinal mastocytosis was also observed with CsA treatment in worm-infected rats [52]. In systemic mastocytosis

patients, CsA combined with glucocorticoid demonstrated good clinicopathological response [53, 54]. Yoko *et al.* [55] successfully applied tacrolimus ointment to treat adult-onset mastocytosis of a 64-year-old woman. The patient received oral administration of leukotriene antagonist and local narrow-band UV-B or tacrolimus ointment. Compared with the UV-B irradiation therapy, topical tacrolimus treatment appeared more preferable. More interestingly, they also found that the number of mast cells infiltrating in the skin lesion was reduced. This observation is consistent with the results in murine cutaneous mastocytosis model treated with pimecrolimus [29], which indicates that topical calcineurin inhibitors may cause deletion of cutaneous mast cells during treatment of mastocytosis. Diffuse cutaneous mastocytosis is a rare form of mastocytosis, in which there are loosely arranged mast cells throughout the dermis, with subepidermal edema leading to vesiculobullous lesions. Bullous mastocytosis is a form of diffuse cutaneous mastocytosis that typically arises during the early stages of life. Tacrolimus 0.1% ointment was used to treat a bullous mastocytosis patient and the improvement was obtained [56].

However, in atopic dermatitis patients or atopic dermatitis animal models the numbers of cutaneous mast cells were not affected by pimecrolimus or tacrolimus treatment, whereas they inhibit the release of mast cell mediators. In atopic dermatitis patients treated with pimecrolimus 1% cream twice daily for three weeks, the dermal infiltration of T cells, B cells and eosinophils were significantly decreased, whereas the numbers of skin mast cells were not changed [57]. Similar results were obtained in atopic dermatitis patients receiving tacrolimus 1% ointment [58]. In a murine atopic dermatitis model, the skin mast cell numbers were not affected by either topical tacrolimus treatment or oral CsA treatment, whereas the ratio of degranulated mast cells was reduced [59]. Moreover, in a murine model of immediate hypersensitivity reactions, the numbers of skin mast cells were not significantly changed with topical tacrolimus, although the percentage of degranulated mast cells decreased in the tacrolimus-treated group. The TUNEL staining indicated that there were no apoptotic mast cells in the foot skin of tacrolimus-treated mice [60]. These results indicate that pimecrolimus and other calcineurin inhibitors seem to have no effects on

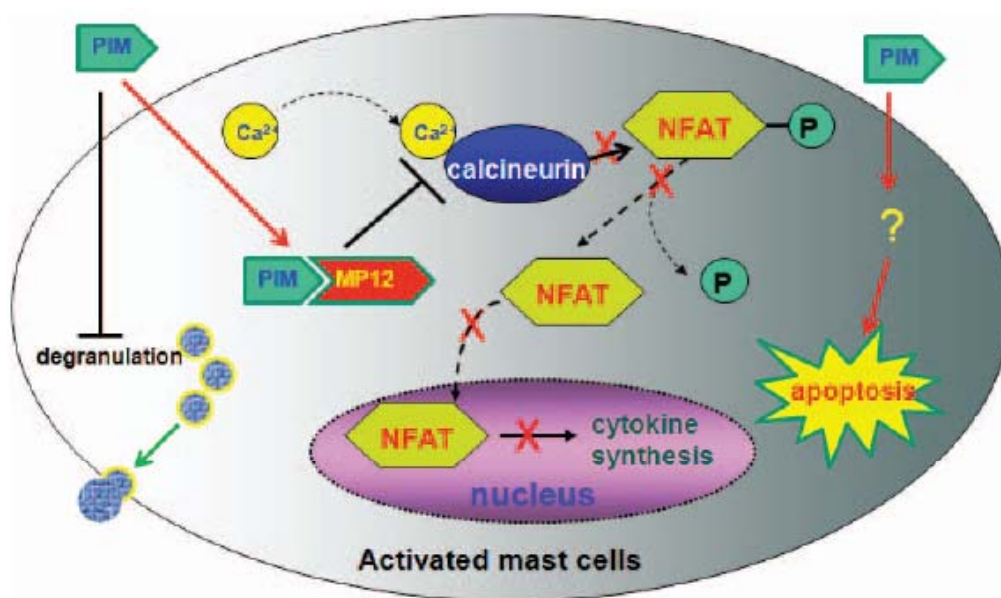


Fig. (2). Mast cell as a target of pimecrolimus (PIM). PIM binds to its specific intracellular receptor macrophilin-12 (MP12) to form inhibitory complexes that block the phosphatase activity of calcineurin, and then the calcineurin-mediated dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NFAT) are blocked, which leads to the inhibition of cytokine synthesis and release. PIM also inhibits degranulation of activated mast cells and induces mast cells apoptosis through unknown mechanisms.

the numbers of mast cells in the above mentioned diseases or disease models, which is in discordance with their effects in mastocytosis. We propose the hypothesis that the observed effects may be explained by differential effects of calcineurin inhibitors hyperproliferative mast cells induced by SCF [30] but not act on the hyperactive mast cells induced by the allergens. Future scaled-up clinical studies should verify, whether the above hypothesis is correct or not.

5. SUMMARY

Mast cells are a specific target of pimecrolimus, and the activities of pimecrolimus on mast cells include inhibition of release and synthesis of mediators, repression of SCF-dependent proliferation/survival, and induction of apoptosis; however, the underlying molecular mechanisms need to be studied thoroughly (Fig. 2). A number of experimental and clinical studies have demonstrated the successful treatment of mastocytosis with pimecrolimus and other topical calcineurin inhibitors, but the more comprehensive basic and large-scale randomized placebo-controlled clinical studies are required to further verify the roles of pimecrolimus in the treatment of mast cell-associated disease, such as mastocytosis and other mast cell-associated leukemia. Finally, it is important to note that the effects and mechanisms of pimecrolimus on mast cells are more complicated than what have been understood, since there are numerous downstream substrates other than NFAT regulated by calcineurin and a number of signal transduction pathways cross-talked with Ca^{2+} /calmodulin/calcineurin pathway [7].

DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

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REFERENCES

- [1] Galli SJ, Tsai M. Mast cells in allergy and infection: versatile effector and regulatory cells in innate and adaptive immunity. *Eur J Immunol* 2010; 40: 1843-51.
- [2] Rao KN, Brown MA. Mast cells: multifaceted immune cells with diverse roles in health and disease. *Ann N Y Acad Sci* 2008; 1143: 83-104.
- [3] Swindle EJ, Metcalfe DD. The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. *Immunol Rev* 2007; 217: 186-205.
- [4] Hamid Q, Tulic M. Immunobiology of asthma. *Annu Rev Physiol* 2009; 71: 489-507.
- [5] Brockow K, Metcalfe DD. Mastocytosis. *Chem Immunol Allergy* 2010; 95: 110-24.
- [6] Stuetz A, Baumann K, Grassberger M, Wolff K, Meingassner JG. Discovery of topical calcineurin inhibitors and pharmacological profile of pimecrolimus. *Int Arch Allergy Immunol* 2007; 142: 255-64.
- [7] Li H, Rao A, Hogan PG. Interaction of calcineurin with substrates and targeting proteins. *Trends Cell Biol* 2011; 21: 91-103.
- [8] Müller MR, Rao A. NFAT, immunity and cancer: a transcription factor comes of age. *Nat Rev Immunol* 2010; 10: 645-56.
- [9] Martínez-Martínez S, Redondo JM. Inhibitors of the calcineurin/NFAT pathway. *Curr Med Chem* 2004; 11: 997-1007.
- [10] Grassberger M, Steinhoff M, Schneider D, Luger TA. Pimecrolimus – an anti-inflammatory drug targeting the skin. *Exp Dermatol* 2004; 13: 721-30.
- [11] Meingassner JG, Kowalsky E, Schwendinger H, Elbe-Bürger A, Stütz A. Pimecrolimus does not affect Langerhans cells in murine epidermis. *Br J Dermatol* 2003; 149: 853-7.
- [12] Hoetzonecker W, Meingassner JG, Ecker R, Stingl G, Stuetz A, Elbe-Bürger A. Corticosteroids but not pimecrolimus affect viability, maturation and immune function of murine epidermal Langerhans cells. *J Invest Dermatol* 2004; 122: 673-84.
- [13] Hoetzonecker W, Ecker R, Kopp T, Stuetz A, Stingl G, Elbe-Bürger A. Pimecrolimus leads to an apoptosis-induced depletion of T cells but not Langerhans cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 2005; 115: 1276-83.
- [14] Hoetzonecker W, Meindl S, Stuetz A, Stingl G, Elbe-Bürger A. Both pimecrolimus and corticosteroids deplete plasmacytoid dendritic cells in patients with atopic dermatitis. *J Invest Dermatol* 2006; 126: 2141-4.
- [15] Hebert AA. Review of pimecrolimus cream 1% for the treatment of mild to moderate atopic dermatitis. *Clin Ther* 2006; 28: 1972-82.
- [16] Wollina U, Hansel G, Koch A, Abdel-Naser MB. Topical pimecrolimus for skin disease other than atopic dermatitis. *Expert Opin Pharmacother* 2006; 7: 1967-75.
- [17] Fabroni C, Lotti T. Pimecrolimus in dermatology. *G Ital Dermatol Venereol* 2009; 144: 321-5.
- [18] Hultsch T, Müller KD, Meingassner JG, Grassberger M, Schopf RE, Knop J. Ascomycin macrolactam derivative SDZ ASM 981 inhibits the release of granule-associated mediators and of newly synthesized cytokines in RBL 2H3 mast cells in an immunophilin-dependent manner. *Arch Dermatol Res* 1998; 290: 501-7.
- [19] Grassberger M, Baumruker T, Enz A, *et al.* A novel anti-inflammatory drug, SDZ ASM 981, for the treatment of skin diseases: *in vitro* pharmacology. *Br J Dermatol* 1999; 141: 264-73.
- [20] Zuberbier T, Chong SU, Grunow K, Guhl S, Welker P, Grassberger M, Henz BM. The ascomycin macrolactam pimecrolimus (Elidel, SDZ ASM 981) is a potent inhibitor of mediator release from human dermal mast cells and peripheral blood basophils. *J Allergy Clin Immunol* 2001; 108: 275-80.
- [21] Sperr WR, Agis H, Czerwenka K, *et al.* Effects of cyclosporin A and FK-506 on stem cell factor-induced histamine secretion and growth of human mast cells. *J Allergy Clin Immunol* 1996; 98: 389-99.
- [22] Narenjkar J, Assem el-SK, Wan BY, Marsh S, Ezeamuzie CI. Effect of cyclosporin and tacrolimus (FK506) on the antigen-induced mediator release, membrane potential and $^{86}\text{Rb}^{+}/\text{K}^{+}$ and Ca^{2+} fluxes in the RBL-2H3 cell line. *Int Immunopharmacol* 2006; 6: 742-9.
- [23] Oh-hora M. Calcium signaling in the development and function of T-lineage cells. *Immunol Rev* 2009; 231: 210-24.
- [24] Sim AT, Ludowyke RI, Verrills NM. Mast cell function: regulation of degranulation by serine/threonine phosphatases. *Pharmacol Ther* 2006; 112: 425-39.
- [25] Zhang J, Mendoza M, Guiraldelli MF, Barbu EA, Siraganian RP. Small interfering RNA screen for phosphatases involved in IgE-mediated mast cell degranulation. *J Immunol* 2010; 184: 7178-85.
- [26] Zhang B, Alysandratos KD, Angelidou A, *et al.* Human mast cell degranulation and preformed TNF secretion require mitochondrial translocation to exocytosis sites: Relevance to atopic dermatitis. *J Allergy Clin Immunol* 2011 Mar 29. [Epub ahead of print]
- [27] Cereggetti GM, Stangherlin A, Martins de Brito O, *et al.* Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc Natl Acad Sci U S A* 2008; 105: 15803-8.
- [28] Ständer S, Ständer H, Seeliger S, Luger TA, Steinhoff M. Topical pimecrolimus and tacrolimus transiently induce neuropeptide release and mast cell degranulation in murine skin. *Br J Dermatol* 2007; 156: 1020-6.
- [29] Ma Z, Tovar JP, Kwong KY, Paek D. Pimecrolimus induces apoptosis of mast cells in a murine model of cutaneous mastocytosis. *Int Arch Allergy Immunol* 2010; 153: 413-8.
- [30] Ito F, Toyota N, Sakai H, Takahashi H, Iizuka H. FK506 and cyclosporin A inhibit stem cell factor-dependent cell proliferation/survival, while inducing upregulation of c-kit expression in cells of the mast cell line MC/9. *Arch Dermatol Res* 1999; 291: 275-83.
- [31] Jensen BM, Akin C, Gilfillan AM. Pharmacological targeting of the KIT growth factor receptor: a therapeutic consideration for mast cell disorders. *Br J Pharmacol* 2008; 154: 1572-82.
- [32] Ullerås E, Karlberg M, Möller Westerberg C, *et al.* NFAT but not NF- κB is critical for transcriptional induction of the prosurvival gene A1 after IgE receptor activation in mast cells. *Blood* 2008; 111: 3081-9.

- [33] Kiani A, Habermann I, Haase M, *et al.* Expression and regulation of NFAT (nuclear factors of activated T cells) in human CD34⁺ cells: down-regulation upon myeloid differentiation. *J Leukoc Biol* 2004; 76: 1057-65.
- [34] Medyouf H, Ghysdael J. The calcineurin/NFAT signaling pathway: a novel therapeutic target in leukemia and solid tumors. *Cell Cycle* 2008; 7: 297-303.
- [35] Canté-Barrett K, Winslow MM, Crabtree GR. Selective role of NFATc3 in positive selection of thymocytes. *J Immunol* 2007; 179: 103-10.
- [36] Buchholz M, Schatz A, Wagner M, *et al.* Overexpression of c-myc in pancreatic cancer caused by ectopic activation of NFATc1 and the Ca²⁺/calcineurin signaling pathway. *EMBO J* 2006; 25: 3714-24.
- [37] Medyouf H, Alcalde H, Berthier C, *et al.* Targeting calcineurin activation as a therapeutic strategy for T-cell acute lymphoblastic leukemia. *Nat Med* 2007; 13: 736-41.
- [38] Pham LV, Tamayo AT, Yoshimura LC, Lin-Lee YC, Ford RJ. Constitutive NF- κ B and NFAT activation in aggressive B-cell lymphomas synergistically activates the CD154 gene and maintains lymphoma cell survival. *Blood* 2005; 106: 3940-7.
- [39] Soleimanpour SA, Crutchlow MF, Ferrari AM, *et al.* Calcineurin signaling regulates human islet β -cell survival. *J Biol Chem* 2010; 285: 40050-9.
- [40] Heit JJ, Apelqvist AA, Gu X, Winslow MM, Neilson JR, Crabtree GR, Kim SK. Calcineurin/NFAT signalling regulates pancreatic β -cell growth and function. *Nature* 2006; 443: 345-9.
- [41] Alvarez S, Blanco A, Fresno M, Muñoz-Fernández MÁ. TNF- α contributes to caspase-3 independent apoptosis in neuroblastoma cells: role of NFAT. *PLoS One* 2011; 6:e16100.
- [42] Shibasaki F, McKeon F. Calcineurin functions in Ca²⁺-activated cell death in mammalian cells. *J Cell Biol* 1995; 131: 735-43.
- [43] Wang HG, Pathan N, Ethell IM, *et al.* Ca²⁺-induced apoptosis through calcineurin dephosphorylation of BAD. *Science* 1999; 284: 339-43.
- [44] Nissi R, Kotila V, Knuuti E, Väre PO, Kauppila S. Altered p53 and Bcl-2 expression in keratinocytes of vulvar lichen sclerosis during pimecrolimus treatment. *Br J Dermatol* 2009; 161: 958-60.
- [45] Metcalfe DD. Mast cells and mastocytosis. *Blood* 2008; 112: 946-56.
- [46] Valent P, Horny HP, Escribano L, *et al.* Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001; 25: 603-25.
- [47] Briley LD, Phillips CM. Cutaneous mastocytosis: a review focusing on the pediatric population. *Clin Pediatr (Phila)* 2008; 119: 194-8.
- [48] Valent P, Akin C, Sperr WR, *et al.* Mastocytosis: pathology, genetics, and current options for therapy. *Leuk Lymphoma* 2005; 46: 35-48.
- [49] Lee HW, Jeong YI, Choi JC, Lee MW, Choi JH, Moon KC, Koh JK. Two cases of telangiectasis macularis eruptive perstans demonstrated by immunohistochemistry for c-kit (CD 117). *J Dermatol* 2005; 32: 817-20.
- [50] Correia O, Duarte AF, Quirino P, Azevedo R, Delgado L. Cutaneous mastocytosis: Two pediatric cases treated with topical pimecrolimus. *Dermatol Online J* 2010; 16: 8.
- [51] Fujino T, Ichikawa H, Fried B. The immunosuppressive compound FK506 does not affect expulsion of *Echinostoma trivolvis* in C3H mice. *Parasitol Res* 1998; 84: 519-21.
- [52] Shin EH, Kim TH, Hong SJ, Park JH, Guk SM, Chai JY. Effects of anti-allergic drugs on intestinal mastocytosis and worm expulsion of rats infected with *Neodiplostomum seoulense*. *Korean J Parasitol* 2003; 41: 81-7.
- [53] Ozdemir D, Dagdelen S, Erbas T, *et al.* Hypotension, syncope, and fever in systemic mastocytosis without skin infiltration and rapid response to corticosteroid and cyclosporin: a case report. *Case Report Med* 2010; 2010: 782595.
- [54] Kurosawa M, Amano H, Kanbe N, *et al.* Response to cyclosporin and low-dose methylprednisolone in aggressive systemic mastocytosis. *J Allergy Clin Immunol* 1999; 103(5 Pt 2): S412-20.
- [55] Yoko N, Yasuyuki S, Hiroyuki M, Chika O, Ichiro K, Tatsuhiko F. Successful treatment with FK506 ointment for adult-onset mastocytosis: a case report. *Jap J Dermatol* 2005; 115: 2232-5.
- [56] Avshalumov K, Pichardo R, Jorizzo JL, Sanguenza OP, Goldenberg G. Bullous mastocytosis: report of a patient and a brief review of the literature. *Am J Dermatopathol* 2008; 30: 455-7.
- [57] Simon D, Vassina E, Yousefi S, Braathen LR, Simon HU. Inflammatory cell numbers and cytokine expression in atopic dermatitis after topical pimecrolimus treatment. *Allergy* 2005; 60: 944-51.
- [58] Simon D, Vassina E, Yousefi S, Kozłowski E, Braathen LR, Simon HU. Reduced dermal infiltration of cytokine-expressing inflammatory cells in atopic dermatitis after short-term topical tacrolimus treatment. *J Allergy Clin Immunol* 2004; 114: 887-95.
- [59] Yamashita H, Ito T, Kato H, Asai S, Tanaka H, Nagai H, Inagaki N. Comparison of the efficacy of tacrolimus and cyclosporine A in a murine model of dinitrofluorobenzene-induced atopic dermatitis. *Eur J Pharmacol* 2010; 645: 171-6.
- [60] Inoue T, Katoh N, Kishimoto S. Prolonged topical application of tacrolimus inhibits immediate hypersensitivity reactions by reducing degranulation of mast cells. *Acta Derm Venereol* 2006; 86: 13-6.