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Mast Cell Stabilisers

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Abstract

Mast cells play a critical role in type 1 hypersensitivity reactions. Indeed mast cell mediators are implicated in many different conditions including allergic rhinitis, conjunctivitis, asthma, psoriasis, mastocytosis and the progression of many different cancers. Thus, there is intense interest in the development of agents which prevent mast cell mediator release or which inhibit the actions of such mediators once released into the environment of the cell. Much progress, into the design of new agents, has been made since the initial discovery of the mast cell stabilising properties of khellin from *Amni visnaga* and the clinical approval of disodium cromoglycate. This review critically examines the progress that has been made in the intervening years from the design of new agents that target a specific signalling event in the mast cell degranulation pathway to those agents which have been developed where the precise mechanism of action remains elusive. Particular emphasis is also placed on clinically used drugs for other indications that stabilise mast cells and how this additional action may be harnessed for their clinical use in disease processes where mast cells are implicated.

Keywords: Mast cell, Sensitisation, Anti-immunoglobulin E, Mediator release, Tyrosine kinases, Allergy, Inhibitors, Disodium cromoglycate, Natural products.

1 Introduction

Mast cells were first described by Paul Ehrlich in 1878, who named them "mastzellen", meaning "feeding cells" because of their appearance (Ehrlich, 1878; Galli et al., 1999). The precursor to mast cells expressing $CD34^+$ molecule are formed in the bone marrow during haematopoiesis and circulate into the bloodstream (Robbie & Brown, 2002). The haematopoietic progenitor cells remain undifferentiated in the blood and become differentiated only upon entering the tissue, where they become mature mast cells under the influence of local factors. Mast cells can be very long-lived, ranging from weeks to months (Wedmeyer et al., 2000). They are found in almost all parts of the body along with the endothelial cells of the blood vessel wall as well as the mucosal epithelial tissue (Nauta et al., 2008). It has been established by many studies that mast cell proliferation, differentiation and survival are strictly regulated by stem cell factor (SCF), which act through its Kit receptor expressed on the mast cell surface (Sundström et al., 2001). Under normal conditions, mast cell numbers in tissue are considered to be relatively constant, except when mast cell hyperplasia is established in different pathologies such as chronic inflammatory processes, fibrotic disorders and wound healing (Bischoff & Sellge, 2002).

Two distinct phenotypes of mast cells are distinguishable based on the types of proteases contained in their exocytotic granules. Mucosal mast cells contain only tryptase (namely M_T), which are mainly found in the mucosa of the gastrointestinal system and in the lamina of the respiratory tract, whereas those found in connective tissue mast cells contain tryptase, chymase, cathepsin G and carboxypeptidase (namely M_{TC}), which are localized in the sub-mucosa of the gastrointestinal tract, skin and peritoneum (Rao, 2002a; Puxeddu et al., 2003).

1.1 Mast cell activation

Mast cells express a vast array of stimulatory and inhibitory receptors. Mast cell activation can be induced by both immunologic (immunoglobulin E (IgE)) and non-immunologic substances. Crosslinking of IgE, immunoglobulin G_1(IgG$_1$), immunoglobulin G_{2a} (IgG$_{2a}$) and immunoglobulin G_{2b} (IgG$_{2b}$) antibodies on the high affinity IgE receptor (FcεRI), the FcγRI (human), FcγRIIa (mouse and human) or FcγRIII (mouse) receptor by allergen incites antigen-specific mast cell activation (Nimmerjahn and Ravetch, 2008).

1.1.1. Mechanism of IgE mediated degranulation-the classic pathway

IgE-dependent activation of mast cells, basophils, monocytes, and macrophages play a vital role in the pathogenesis of allergic reactions (Maurer et al., 1994; Sutton et al., 2000; Owen, 2002). IgE is the major antibody in allergic diseases. The main physiological role of IgE is believed to be to protect the external mucosal surface of the body by the local recruitment of plasma factors and effector cells, by inducing an acute inflammatory reaction (Rao, 2002e). IgE is largely cell bound, especially to mast cells. Mast cell degranulation results from antigen cross-linking of IgE on cell surface receptors.

1.1.2. Steps involved in FcεRI signal transduction leading to mast cell degranulation

An enzyme of the Src family of tyrosine kinases, called Lyn is constitutively associated with the β-chain of the FcεRI. Upon cross-linking of IgE bound to the α-subunits of FcεRI, Lyn phosphorylates a special amino acid sequence called ITAM (immunoreceptor tyrosine-based activation motif) that is located on both β and γ-chains of FcεRI. Phosphorylation of the β-chain recruits more Lyn, which can associate by its SH2 domain (Src homology domain 2), a region of the Src-family protein that binds phosphorylated tyrosine-containing sequences. Through this mechanism the signal transduction is magnified. Phosphorylation of the γ-chain provides a binding site for the cytoplasmic tyrosine kinase, Syk (spleen tyrosine kinase), which upon phosphorylation by Lyn, and possibly through autophosphorylation, becomes activated. Inhibitory signals to these processes are also present, in the form of signal regulatory proteins (SIRPs), which cause downregulation through catalysis of dephosphorylation reactions. Activated Syk phosphorylates kinases including PI_3 (phosphoinositol-3-OH-kinase), which results in generation of PIP_3 (phosphatidylinositol [3,4,5] triphosphate), and its association with another tyrosine kinase, Btk (Bruton's tyrosine kinase) allows association of Btk with the plasma membrane. Other enzymes thought to be important following Syk activation include MAP kinase (mitogen-activated protein kinase), protein kinase C and Phospholipase C γ (PLC-γ). Upon Btk activation, PLC-γ is phosphorylated by Syk and generates inositol trisphosphate (IP_3) which causes Ca^{2+} release from the endoplasmic reticulum (ER). Depletion of intracellular Ca^{2+} stores activates store-operated calcium channels (SOCC) and allows Ca^{2+} influx. This is aided by increased membrane fluidity through methylation of phospholipids. Ca^{2+} influx promotes activity within the cytoskeleton, causing microtubule assembly and contraction of microfilaments.

Disassembly of actin-myosin complexes facilitates granule contact with the cell membrane. FcεRI activation also results in a rise in cyclic adenosine monophosphate (cAMP), which mediates phosphorylation of the granule membrane proteins, altering their permeability. Swelling of the granules aids fusion of granule and plasma membranes, followed by release of granule contents (Turner and Kinet, 1999).

However, the classical pathway doesn't explain all allergic responses. For example, active systemic anaphylaxis developed in mice that were deficient in mast cells, IgE, or FcεRIα chain (Strait et al., 2002). Various studies have demonstrated the existence of other major distinct pathways resulting in allergen-triggered systemic anaphylaxis, which are mediated by basophils, IgG, IgG receptor, and platelet-activating factor (PAF) (Tsujimura et al., 2008). It has been demonstrated that mast cells can also be activated by neuropeptides released by neuronal stimulation during stress, which include corticotropin-releasing factor (CRF), urocortin (Ucn), nerve growth factor (NGF), substance P (SP), neurotensin (NT) (Suzuki et al., 1999; Theoharides et al., 2004). Activated mast cells secrete vasoactive, pro-inflammatory and neurosensitizating molecules which interact with keratinocytes, endothelial cells or nerve endings, resulting in chronic inflammation and neuropathic hypersensitivity or pain. It has been shown that such mast cell activations can either be mediated through specific receptors (i.e. NK$_1$ receptors), or by activating G protein directly, which is receptor-independent (Theoharides & Cochrane, 2004). Several agents such as opioids and physical stimuli also activate mast cells and they do this independently of IgE (Machado et al., 1996). Moreover, Der p 1 (a major house dust mite allergen) and bee venom phospholipase A2 (major allergen in bee sting allergy), have been shown to induce mast cell activation independently of the classical pathway (Machado et al., 1996). Interestingly, studies showed that certain stimulators could induce biological mediators' release (i.e. histamine, IL-4, TNF) from unsensitized mast cells in the absence of detectable changes in intracellular Ca^{++} such as TSL-1 antigens from *Trichinella spiralis* muscle larvae (Arizmendi-Puga et al., 2006).

2   Sources of mast cell stabilisers

As with the discovery of many medicines in clinical use today, natural resources have served as a very fruitful source of mast cell stabilisers. Invariably, preparation of semi-synthetic derivatives of the natural "hits" from screening assays resulted in the generation of more potent derivatives. Purely synthetic compounds have been prepared to target specific enzymes or receptors involved in degranulation. Oftentimes these compounds were designed

to target other conditions but as a consequence of their action they have the knock-on effect of also stabilising mast cells. Promising results have been generated using antibodies with the human mAb omalizumab now in clinical use for the treatment of asthma, Figure 1.
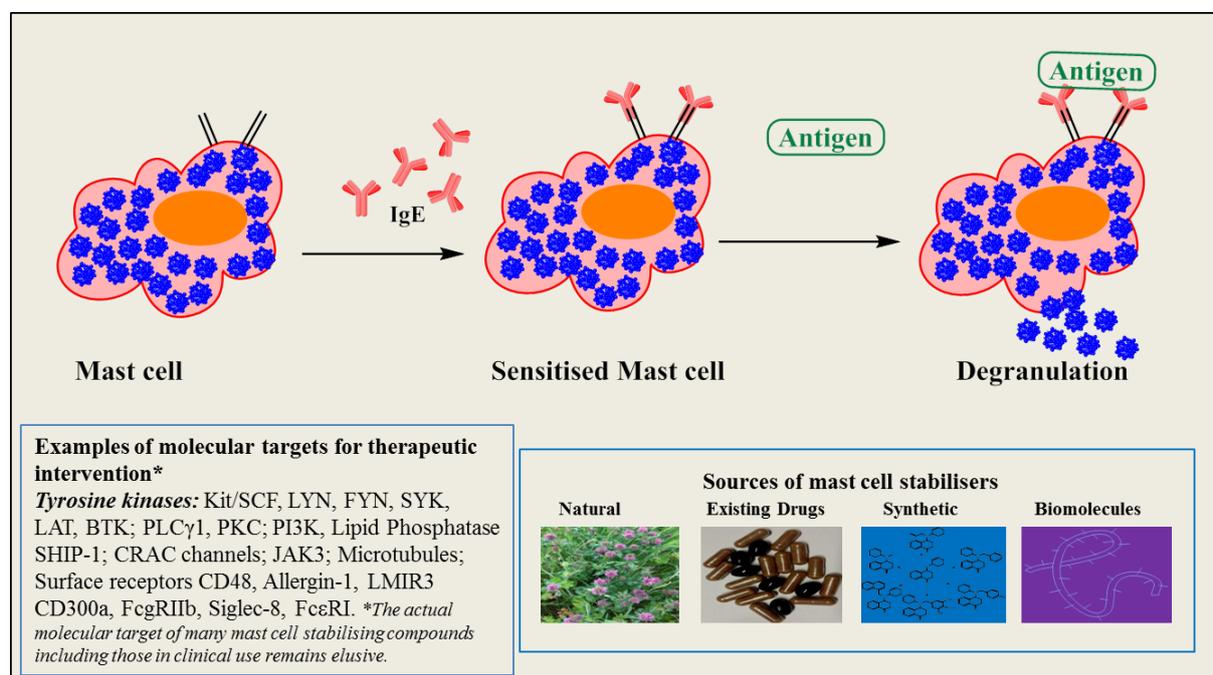

Figure 1. Mast cell sensitisation, degranulation, possible molecular targets for therapeutic intervention and sources of mast cell stabilisers.

2.1 Clinically approved mast cell stabilizers

2.1.1 Disodium cromoglycate

In 1967, following fortuitous studies on compounds with structural similarity to khellin, disodium cromoglycate (DSCG) was launched as the first of a new therapeutical class, the mast cell-stabilisers (Altounyan, 1967). Disodium cromoglycate can inhibit both passive cutaneous anaphylaxis (PCA) in the rat and the release of histamine from rat peritoneal cells induced by Compound 48/80 (Orr et al., 1971). Using enzymatic and mechanical methods of dispersion, Church *et al.* demonstrated that disodium cromoglycate and similar anti-allergic drugs caused weak inhibition (<35% at 100-1000μM) of histamine release from human lung cells, although all anti-allergic drugs tested, unlike the more potent β-agonist, salbutamol, showed rapid development of tachyphlaxis (Church and Gradidge, 1980). Interestingly, in this study, disodium cromoglycate was also shown to offer effective inhibition of prostaglandin D_2 release from lung mast cells. Using human lung cells obtained by bronchoalveolar lavage rather than from lobectomy specimens, Leung *et al*. demonstrated a

dose-dependent inhibition of anti-IgE-induced histamine release using both disodium cromoglycate and nedocromil (Leung et al. 1988). In a comparison with collagenase-digested parenchymal lung tissue, Leung showed the phenomenon of tachyphlaxis to occur only in the former preparation, which may partly explain the usefulness of these drugs in clinical practice.

To this day, the precise mode of action of these mast cell-stabilising molecules is still the subject of debate, and many additional theories have been forwarded, including tachykinin antagonism and inhibition of tumour necrosis factor α (TNFα) release from mast cells (Edwards and Norris, 1994) and the G-Protein-Coupled Receptor 35 (Yang et al., 2010). Despite the fact that disodium cromoglycate is not therapeutically effective in all patients, and has mainly an adjunctive role in asthma, its excellent safety profile has made it especially useful in this disease, especially in children. It also has a therapeutic role in diseases such as mastocytosis and allergic conjunctivitis.

2.1.2   Nedocromil

Nedocromil sodium, a pyrano quinoline, is also indicated as a prophylactic in asthma (Brogden and Sorkin, 1993). Like disodium cromoglycate, nedocromil sodium produces a dose-dependent inhibition of histamine secretion from human pulmonary mast cells, but is one order more potent. The heterogeneity of mast cells was further emphasised in a study by Louis et al who demonstrated the anility of nedocromil to prevent anti-IgE or substance P-induced histamine release from chopped lung, but not from basophils or sliced skin (Louis and Radermecker, 1990). Unlike disodium cromoglycate, when administered to *Ascaris*-sensitive monkeys, nedocromil inhibited the bronchoconstriction caused by antigen challenge, and further prevented histamine release from bronchoalveolar lavage cells (Eady et al, 1985).

2.1.3   Lodoxamide

Lodoxamide [*N',N'*-(2-choloro-5-cyano-m-phenylene)dioxamic acid] is another clinically effective mast cell stabiliser. It showed significantly greater inhibition of mediator release than DSCG by several routes of administration (i.e. inhalation, oral, intraperitoneal injection, intravenous injection) in animal models of asthma (i.e. rats, primates), especially, 2,500 times more active than DSCG (ID50 = 0.001 mg/kg) shown in rat studies (Johnson & Sheridan,

1986). The salt form of lodoxamide, namely lodoxamide tromethamine [*N',N*'-(2-chloro-5-cyano-m-phenylene) dioxamic acid tromethamine salt}, has been successfully developed. In this presentation, it was shown to be safe and effective for treatment of ocular allergic diseases (Avunduk et al., 2000). A comparative study demonstrated that lodoxamide tromethamine was more efficacious than DSCG in rabbits with passive allergic conjunctivitis (Özturk et al., 2002).

2.1.4   Ketoifen, Olopatadine, Azelastine and Epinastine

These drugs belong to the dual class of anti-allergic drugs which possess H1 antagonist activity as well as mast cell stabilising properties.

2.1.4.1   Ketotifen

Among the antihistamines, the cyproheptadine analogue ketotifen possesses an additional action on mast cells similar to disodium cromoglycate. It is claimed that the action on mast cells can be dissociated from, and is independent of, its anti-histaminic properties, but may be linked to its ability to inhibit reactions to platelet activating factor (PAF) (Grant et al, 1990)[i]. It has a similar clinical applicability to disodium cromoglycate; both drugs show comparable reductions in the need for adjunctive therapies. Unlike disodium cromoglycate, ketotifen is orally active.

2.1.4.2   Olopatadine

Karaman et al., (2008) showed that olopatadine hydrochloride was more effective in patients with acute than in those with chronic allergic disorders, without demonstrating major detectable side effects. Olopatadine hydrochloride ophthalmic solution 0.1% (Opatanol) is widely used in clinical treatment of ocular allergic diseases. It shows a rapid onset of action in the treatment of allergic conjunctivitis (Abelson & Spitalny, 1998). Recently, a 0.2% olopatadine hydrochloride ophthalmic solution (Pataday) has been proven to be the first effective ophthalmic antiallergy drug with once-daily dosing (Abelson & Gomes, 2008). Several comparative studies showed that different concentrations of olopatadine hydrochloride nasal spray (0.1%, 0.2%, 0.4%, 0.6%) were efficacious in suppressing allergic reactions by reducing total allergen-induced nasal symptoms in comparison to placebo nasal spray. Olopatadine at 0.2% concentration inhibited mast cell degranulation and at 0.6% resulted in remarkably fast onset as well as long lasting action (Sanico et al., 2004; Patel et

al., 2007; Pipkorn et al., 2008). An intranasal formulation of olopatadine hydrochloride (Patanase, olopatadine hydrochloride nasal spray 0.6%) has been developed to treat allergic rhinitis (Saltoun & Avila, 2008). Suppression of increased levels of mast cell derived NGF and VEGF, as well as histamine H1 receptor antagonism account for olopatadine's therapeutic effects in the treatment of allergic inflammations (Tamura & Komai, 2008).

2.1.4.3 Azelastine

Azelastine hydrochloride has been extensively studied and has been shown to possess anti-allergic and anti-inflammatory activities *in vivo* as well as *in vitro*. Many observations were reported upon evaluation of azelastine, including superior inhibition of IL-6, tryptase and histamine secretion from mast cells over olopatadine (Kempuraj et al., 2002 and Kempuraj et al., 2003).

2.1.4.4 Epinastine

Epinastine hydrochloride was first approved for the treatment of rhinitis in 1981 (Finegold et al., 2006). In a randomised, double-blind, crossover study, (Torkildsen et al., 2008) concluded that epinastine was more comfortable for patients with allergic conjunctivitis following a single drop administration compared to azelastine and ketotifen.

3   Clinically approved drugs for other indications with mast cell stabilising properties

The design of drugs with target specificity represents a significant challenge. Thus, it is not surprising that many of the clinically approved drugs also have several off-target interactions. Alternatively, their on-target activity may also have consequences for conditions unrelated to their initial indication. In this context, several classes of approved drugs, for indications outside of the allergy domain, also exhibit mast cell stabilising properties, including the statin class of HMGCoA reductase inhibitors, the second generation tyrosine kinase inhibitors, nilotinib, sunitinib, ibrutinib and dasatinib, the mucolytic agent, ambroxol, loop diuretic, fusemide, L-type Ca^{++} channel blockers including verapamil and nifedipine, the phosphodiesterase inhibitor, theophylline and the immunomodulatory agents, cyclosporine and FK506 (tacrolimus).

3.1 Anti-hypercholesterolemia drugs

The statin class is best represented by cerivastatin, atorvastatin and fluvastatin all of which have shown varying degrees of mast cell stabilisation against anti-IgE mediated release of histamine from different mast cell populations. Cerivastatin and atorvastatin prevented anti-IgE-induced histamine release from mature lung mast cells in a dose-dependent manner, while they also suppressed cytokine-dependent growth of normal mast cell progenitors in HMC-1 cells. Taken together this data suggests that cerivastatin and atorvastatin are inhibitors of mast cell growth and function (Krauth et al., 2006). Using RBL-3H2 cells, fluvastatin inhibited antigen-induced degranulation in a concentration dependent manner (0.5-10 µM) without affecting cytosolic calcium levels or the granule content of these cells. It is hypothesised that the inhibitory action of fluvastatin may be centred on the suppression of geranylgeranyl transferase *via* the depletion of intracellular mevalonic acid (Fujimoto et al., 2009).

3.2 Anti-cancer

3.2.1 Nilotinib

The second-generation KIT tyrosine kinase inhibitor, nilotinib, which is used for the treatment of imatinib resistant BCR-ABL positive chronic myelogenous leukemia, has also shown mast cell stabilising effects. It dose-dependently (5-20 µM) inhibited histamine release induced by compound 48/80 or ovalbumin from rat peritoneal mast cells (RPMCs) and also reduced the secretion of pro-inflammatory cytokines as well as TNF-α expression in RPMCs. This effect was translated *in vivo* where administration of nilotinib prevented systematic anaphylaxis in mice mediated by ovalbumin in a dose-dependent manner (25, 50 and 75 mg/kg, oral mg/kg) (El-Agamy et al., 2012). Midostaurin (PKC412) is a tyrosine kinase inhibitor which interacts with Kit and protein kinase C on mast cells and is used in clinical trials to counteract the growth of neoplastic mast cells in mastocytosis. It inhibited anti-IgE-induced mediator release in blood basophils and cultured cord blood cell-derived mast cells in all samples examined using both the basophil and mast cell lines, KU812 and HMC-1 in a dose-dependent manner at low concentrations (1-1000 nM) (Krauth et al., 2009).

3.2.2 Sunitinib

Sunitinib, a multi-targeted receptor tyrosine kinase inhibitor, is also clinically used for the same indication as nilotinib as well as for renal cell carcinoma. It has been evaluated as a potential anti-allergic agent (Yamaki K., et al., 2012). Daily administration of sunitinib throughout antigen challenge prevented oral antigen-induced anaphylaxis including diarrhea, anaphylactic symptoms, and hypothermia. It also greatly reduced IgE-dependent degranulation and growth of rat basophilic leukemia RBL2H3 and bone marrow-derived mast cells (BMMCs).

3.2.3 Ibrutinib

Ibrutinib has gained recent clinical approval for the treatment of mantle cell lymphoma and chronic lymphocytic leukemia (Small, 2013). Central to its mechanism of action is the manner in which it covalently attaches, in a non-reversible manner, to Cys-481 in the active site of Bruton's tyrosine kinase (Btk) and thus prevents phosphorylation of Tyr-228. Btk is a member of the Tec family tyrosine kinase which is the second largest family of cytoplasmic tyrosine kinases. Btk is significantly expressed on B-cells and also on myeloid cells including mast cells. Studies have also shown that it potently inhibited histamine and PGD2 release from mast cells following crosslinking with anti-IgE antibodies as well as the PCA reaction in a murine model. While ibrutinib, also referred to as PCI-32765, has shown some potential for arthritic treatment, there are likely to be significant side effects associated with its use as it is known to inhibit 22 tyrosine kinases other than Btk at nanomolar concentrations and does so through non-reversible covalent interaction. In this context, CC-292 (N-(3-(5-fluoro-2-(4-(2-methoxyethoxy)phenylamino)pyrimidin-4-ylamino)phenyl)acrylamide), a highly selective Btk inhibitor with a similar mechanism of action to ibrutinib has successfully completed early stage pharmacodynamics assessment in humans (Evans et al., 2013).

3.2.4 Dasatinib

Dasatinib, while targeting Btk and KIT, does so by a non-covalent interaction. It has been demonstrated to inhibit FceRI degranulation in basophils and allergen sensitised individuals (Kneidinger et al., 2008). It is used clinically to treat chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia.

3.3 Mucolytic

At very high concentrations, the mucolytic agent, ambroxol, was shown to inhibit histamine release by more than 50% from human adenoidal mast cells (1 mM) stimulated by concanavalin A and from skin mast cells (100 µM) stimulated by compound 48/80. At 100 µM, it also inhibited anti-IgE induced release of histamine, LTC_4, IL-4 and IL-13 from basophils (Gibbs et al., 1999).

3.4 Diuretic

Frusemide, a loop diuretic, dose-dependently inhibited histamine release from RPMCs within a concentration of $10^{-3} – 10^{-5}$ M stimulated by a various secretagogues known to increase the concentration of intracellular calcium. Interestingly, another loop diuretic, bumetanide, did not have the same effects on rat peritoneal mast cell degranulation suggesting that its mode of inhibition is not mediated via its diuretic $Na+/K+/Cl^-$ co-transporter capacity (Stenton and Lau 1996). The authors observed a similar inhibition profile to that of DSCG with the elicitors used to mediate mast cell degranulation.

3.5 Hypertension

Clinically, dihydropyridines (DHPs) have been used to treat mild to moderate hypertension (Kawabe et al., 2008), angina, atrial arrhythmia, and myocardial ischaemia by inhibiting transmembrane influx of Ca^{++} through L-type Ca^{++} channel, and as such inciting vascular smooth muscle relaxation, and the repression of cardiac contractility (Struyker-Boudier et al., 1990). Some DHP derivatives including nifedipine and verapamil have been developed and found to possess anti-allergic activity acting as mast cell stabilisers, including inhibitory effects on histamine release induced by calcium ionophore A23187 and antigen induced challenge to RPMCs (Chand et al., 1984).

3.6 Immunomodulation

Effective inhibition of IgE-dependent mast cell activation was also observed for immunomodulatory agents. These include FK506 (tacrolimus) (Sengoku et al., 1999) and cyclosporine (Harrison et al., 2007), both of which are commonly used to treat autoimmune diseases, prevention of organ rejection following transplantation and severe allergic conjunctivitis. It has been suggested that the mast cell-stabilising abilities of FK506 and

cyclosporine are modulated by different mechanisms, as the ability of cyclosporine, but not FK506, to inhibit Ca^{2+}-dependent protein phosphatase activity queries whether FK506 stabilizes mast cells by interacting with calcineurin (Harrison et al., 2007).

3.7 Bronchodilator

Phosphodiesterases (PDEs) are a class of enzyme hydrolysing cyclic nucleotide, which are central to their role in modulating cell functions by regulating intracellular levels of cAMP and cGMP. As PDE activity is observed in almost every cell in the body, PDE has been suggested to be a novel target for treatment (Boswell-Smith et al., 2006). Many selective PDE inhibitors have been designed, developed and studied for their anti-allergy and anti-inflammatory properties in a variety of diseases, for example the use of PDE2 inhibitors in sepsis; PDE4 inhibitors in asthma, allergic rhinitis, multiple sclerosis, psoriasis and PDE5 inhibitors in sexual dysfunction in females, cardiovascular disease, and pulmonary hypertension (Boswell-Smith et al., 2006). Some PDE inhibitors have been found to possess mast cell stabilising activity (Weston et al., 1997). Theophylline (1,3-dimethyl-7H-purine-2,6-dione) has been long used to treat asthma because of its anti-inflammatory and bronchodilator activities, as well as its ability to increase diaphragm contractility. In comparison with Nedocromil sodium, theophylline showed significant inhibition of histamine release induced by anti-IgE, compound 48/80 or substance P from human basophils, lung and skin fragments (Louis & Radermecker, 1990).

3.8 Anti-depressant

Although no longer undergoing clinical development as an anti-depressant, the PDE-4 inhibitor, rolipram was shown to dose-dependently (1-100 µM) inhibit histamine release from anti-IgE induced RPMCs and this effect was enhanced when combined with the PDE 3 inhibitor, siguazodan. The level of inhibition of rolipram at 10 µM was $29.4 \pm 7.1\%$ which was elevated in the presence of 1 µM siguazodan to $49.5 \pm 7.1\%$ (Lau and Kam, 2005).

3.9 Anti-malarial

The approval of artesunate for the treatment of malaria represented a welcome digression from the traditional aminoquinoline based anti-malarials where resistance is widespread. This semi-synthetic derivative, derived from the naturally occurring sesquiterpene lactone, artemisinin, has recently been investigated for its potential anti-allergic properties in animal

models of IgE-dependant anaphylaxis (Cheng C et al., 2013). At the mechanistic level, artesunate was shown to prevent IgE-induced Syk and PLCγ1 phosphorylation, formation of IP(3) and rise in cytosolic Ca^{2+} level in mast cells. It also blocked IgE-mediated degranulation of RBL-2H3 mast cells and human culture mast cells. *In vivo*, artesunate prevented IgE-mediated cutaneous vascular hyperpermeability, hypothermia, rise in plasma histamine level, and tracheal tissue mast cell degranulation in mice in a dose-dependent manner.

4   Targeting signalling pathways involved in mast cell degranulation

Aside from targeting Kit and Btk, a significant body of work has been published on targeting Spleen tyrosine kinase (Syk) as a strategy to inhibit mast cell degranulation.

4.1   Spleen tyrosine kinase inhibitors

Syk is a soluble, cytosolic, non-receptor protein tyrosine kinase expressed mainly in most hematopoietic cells, including mast cells, neutrophils, macrophages, B cells, dendritic cells, and epithelial cells (Beaven & Baumgartner, 1996; Miller et al., 2009). It has been shown that Syk is involved in the activation and degranulation of mast cells, lipid mediator synthesis and cytokine production through FcεRI-mediated signalling (Costello et al., 1996). In addition, Syk plays a critical role in signal transduction for immunoreceptors for IgG (FcγR). It is generally believed that Syk is strongly associated with a variety of allergic disorders, antibody-mediated autoimmune diseases as well as certain cancers (Li et al., 2009). Accordingly, Syk kinase inhibition has become an attractive therapeutic intervention. Structurally, there are many classes of Syk kinase inhibitors; from pyrimdine types, those based on an acridone backbone to isoquinolin-10-ones.

4.1.1   Pyrimidine class

In the pyrimidine class, R406 [N 4-(2,2-dimethyl-3-oxo-4H-pyrid[1,4]oxazin-6-yl)-5-fluoro-N2-(3,4,5-trimethoxyphenyl)-2,4-pyrimidinediamine] inhibited phosphorylation of Syk substrate linker for activation of T cells in mast cells and B-cell linker protein/SLP65 in B cells. It also dose-dependently inhibited anti-IgE-mediated cultured human mast cells (CHMC) degranulation, but not ionomycin induced release suggesting that R406 inhibition of mediator degranulation is specific to FcR signalling (Braselmann et al. 2006). BAY61-3606 {2-[7-(3,4-dimethoxyphenyl)-imidazo[1,2-c] pyrimidin-5-ylamino]-nicotinamide dihydrochloride} is a

potent and selective Syk kinase inhibitor which showed significant suppression of antigen-induced passive cutaneous anaphylactic reaction, bronchoconstriction and bronchial edema in addition to reduction of antigen-induced airway inflammation in the rat (Yamamoto et al., 2003). Another potent Syk kinase inhibitor, namely R112, from the 2, 4-diaminopyrimidine class completely and rapidly inhibited histamine release in allergen-induced basophils in addition to lipid mediator and cytokine production of CHMC stimulated by allergen. Its mechanism of action was mediated through inactivation of Syk which consequently prevented downstream phosphorylation of LAT (Y191). LAT phosphorylation is central to the signalling cascade that leads to mass cell degranulation (Rossi et al., 2006). In clinical trials, treatment with R112 rapidly improved the symptoms of allergic rhinitis in hypersensitive individuals (Masuda and Schmitz 2008). A series of pyrimidine-5-carboxamide derivatives, as novel Syk kinase inhibitors, have been synthesised and evaluated. The class of 4-anilionpyrimidine-5-carboxamides was shown to possess good selectivity for Syk. One of the derivatives, 2-(2-aminoethylamino)-4-{3-(trifluoromethyl)phenylamino}pyrimidine-5-carboxamide, exhibited significant inhibition of the PCA reaction in mice following subcutaneous administration (Hisamichi et al., 2005).

4.1.2 Acridone class

ER-27317, an acridone related-compound, inhibited mast cell response by preventing the phosphorylation and activation of Syk kinase. *In vitro*, it inhibited degranulation in a dose-dependent manner in RBL-2H3 cells, RPMCs and HCMCs, all stimulated by antigen with almost complete inhibition observed in these cell populations at 30 µM. It is hypothesised that ER-27317 selectively interferes with FcɛRIγ phospho-ITAM activation of Syk thus preventing the ensuing signalling cascade (Moriya et al., 1997).

4.1.3 Isoquinolin-10-one class

Recent studies have shown that 3-butyl-1-chloro-8-(2-methoxycarbonyl)phenyl-5*H*-imidazo[1,5-*b*]isoquinolin-10-one (U63A05) dose-dependently inhibited degranulation of RBL-2H3 cells and BMMCs stimulated by antigen across a concentration range of 1-10 µM. This compound also suppressed the secretion of proinflammatory cytokines. *In vivo* studies with U63A05 showed that it suppressed antigen-stimulated PCA reaction in mice at doses ranging from 10-100 mg/kg (Kim do et al., 2011).

4.1.4 Flavonoid class

Flavonoids, isoflavonoids and homoisoflavanoids represent a rich source of naturally occurring pharmacologically active compounds with a broad spectrum of activity including mast cell stabilising (Finn and Walsh, 2013). In this context, homoisoflavanone, which has previously been demonstrated to exhibit anti-angiogenic activity, was evaluated for its anti-allergic properties. It was shown to downregulate PGD2, LTB4, and LTC4 production and also inhibited the biosynthesis of pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α in PMA/A23187-or IgE/antigen-stimulated mast cells. At the molecular level, this compound was shown to inactivate Syk signalling and expression of cPLA2. These *in vitro* effects were translated *in vivo* where homoisoflavanone inhibited IgE-mediated PCA and compound 48/80-induced ear swelling in mouse models (Lee et al., 2014). Morin, a flavonol, prevented the degranulation and the production of cytokines such as TNF-α and IL-4 in both RBL-2H3 cells and BMMCs stimulated by antigen at low concentrations (1-10 µM). Morin demonstrated inhibition of activating phosphorylation of Syk, although parallel *in vitro* kinase studies indicated that morin suppresses the IgE-mediated allergic response by primarily inhibiting Fyn kinase in mast cells. *In vivo*, this flavonol suppressed IgE-mediated PCA in mice almost completely at a dose of 100 mg/kg (Kim et al., 2009).

4.1.5 Anthraquinone class

Anthraquinones have a similar flat structure to that of the flavonoids and usually contain a "phenolic component" to their structure. It is thus not surprising that this class of natural products also have quite diverse pharmacological properties and in many cases multiple mechanisms may be operating to give the overall effect at the cellular level. This is a case in point with emodin (1,3,8-trihydroxy-6-methylanthraquinone). It exerts its mass cell stabilising effect by inhibiting both Syk phosphorylation in the proximal step reaction from the FcεRI as well as the phosphorylation of PKC–IKK2–SNARE complex in the late-stage pathway. Emodin, which has been isolated from Frangula, is known to exhibit anti-bacterial, anti-inflammatory, anti-cancer to anti-allergic effects (Kim et al., 2014).

4.1.6 Xanthone class

The xanthones; mangostin-α, -β and -γ isolated from the pericap of *Garcinia mangostana* L. inhibited the release of histamine from IgE-sensitised RBL-2H3 cells in response to antigen

through suppression of the signalling transduction pathway involving Syk and PLCγ (Itoh et al., 2008).

5 Janus kinase 3 inhibitors

Janus kinase 3 (JAK3) is a protein tyrosine kinase abundantly expressed in mast cells and plays an important role in the FcεRI-mediated mast cell inflammatory response. Studies with mast cells derived from JAK3-null mice, obtained following specific disruption of Jak3 gene in embryonic stem cells, resulted in substantially reduced amounts of granule-associated or newly synthesized inflammatory mediators in response to IgE (D'Cruz and Uckun 2007). JAK3 is activated by cytokines such as IL-2, IL-4, IL-7 and IL-9 upon mast cell activation. This causes phosphorylation and dimerisation of STAT 5A for transcription of target genes involved in inflammation (D'Cruz and Uckun 2007). A selective inhibitor of JAK3, 4-(4`-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline (WHI-131) inhibited calcium ionophore A23187 induced- and IgE/antigen induced-degranulation of RBL-2H3 cells in a concentration-dependent manner. WHI-131 also prevented the release of the lipid mediator LTC$_4$ and the proinflammatory cytokine TNF-α and it also prevented the PCA reaction in mice by blocking degranulation *in vivo* (Malaviya et al., 1999). However, later studies indicated that degranulation of JAK3 deficient BMMCs from mice were inhibited by WHI-131 to the same extent as wild-type mice which implies that WHI-131 has other underlying mechanisms of mast cell stabilisation (Linwong et al., 2005). This was confirmed by Linwong et al who showed that WHI-131 inhibits the PI3K pathway by preventing the antigen-induced activation of Fyn, thus inhibiting the antigen-induced degranulation and phosphorylation of MAPKs in mast cells.

6 Neurokinin 1 receptor antagonists

Substance P present in skin mast cells is considered to play a role in stress-induced inflammatory skin disease such as psoriasis. Studies with CP99994, a substance P NK-1 antagonist, prevented stress-induced response mast cell mediator release when Sprague Dawley rats were treated peripherally at (1 and 2 mg/kg) or intracerebroventricularly (5, 10 and 20 µg). The overarching conclusion from this study suggests that NK-1 antagonists may be used therapeutically to treat stress-induced inflammatory skin diseases provided due consideration is given to the dose used (Erin et al., 2004).

7 Targeting phosphoinositide 3-kinases (PI3K)

Inhibition of P13K enzymes have emerged as attractive targets for the design of novel anti-allergy agents, especially the δ and γ isoforms. These are required for the phosphorylation of phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-triphosphate. The δ isoform is a key participant in the recruitment and activation of certain inflammatory cells and also contributes to allergen-IgE-induced mast cell activation and vascular permeability. Several inhibitors of this isoform have been designed including IC87114, a purine-quinazoline derivative which was shown to reduce allergic airway inflammation and hyperresponsiveness in an ovalbumin sensitised murine asthma model (Lee et al., 2006). IPI-145, which shares some structural similarities to IC87114 is a dual acting inhibitor of δ and γ isoforms of PI3K and is in in phase II trials for allergic asthma and severe rheumatoid arthritis (Okkenhaug, 2013).

8 Targeting SHIP-1 activation

A significant number of novel mast cell stabilisers developed to date exhibit their effects by targeting one or more tyrosine kinase or their receptors. An alternative approach is to target key enzymes involved in the termination of the signalling pathways such as phosphatases. In this regard, significant progress has been made by targeting the lipid phosphatase SHIP-1. This enzyme is known to dephosphorylate the inositol ring of phosphatidylinositol 3,4,5-triphosphate to phosphatidylinositol 3,4-diphosphate. Mast cell degranulation studies with AQX-1125, a compound which has been shown to increase the catalytic activity of SHIP-1, clearly demonstrated that it inhibited degranulation of SHIP1$^{+/+}$ but not SHIP1$^{-/-}$ bone marrow mast cells, albeit a relatively high concentration of 60 μM using DNP-HSA to elicit release (Stenton et al., 2013).

9 Mast cell stabilising agents from natural sources

9.1 Flavonoids

Flavonoids comprise a diverse group of benzo-γ-pyrone derivatives, differing in redox state and/or substitution patterns. Within the flavone class, the most active mast cell stabilisers are luteolin, disometin, myricetin and apigenin, which differ only in substitution of the 2-phenyl moiety. Luteolin inhibited IgE-invoked mediator release from human mast cells in a concentration dependent manner (Kimata et al., 2000b). Later studies using a variety of

different conditions to elicit mass cell degranulation also confirmed the mast cell stabilising properties of luteolin (Kempuraj et al., 2008; Asadi et al., 2010; Asadi and Theoharides, 2012).

All three flavones have inhibited cytokine production and release following basophil challenge with immunological or other cytokine provocation (Mastuda et al., 2002, Hirano et al., 2006). Flavonols, a group of 3-hydroxy flavonoids, have demonstrated anti-allergic activity. Examples include kaempferol, fisetin, quercetin and myricetin. Kaempferol, fisetin and quercetin inhibited histamine release induced by multiple stimuli from RBL-2H3 cells. Additionally, fisetin and quercetin decreased gene expression and production of proinflammatory cytokines (Park et al., 2008). Fisetin was also shown to decrease gene expression of IL-4, inhibit the phosphorylation of various kinases, and suppress the activation of nuclear factor (NF)-κB (Park et al. 2007). Quercetin and kaempferol inhibited the secretion of mediators from RBL-2H3 cells and suppressed the mRNA expression of CD23 and p38 MAPK activation in Caco-2 cells stimulated by IL-4 (Lee et al., 2010). These flavonols also significantly inhibited the release of histamine and cytokines from cultured human mast cells and decreased the elevation of intracellular Ca^{2+} (Kempuraj et al., 2005). Quercetin has been shown to down-regulate the mRNA transcription of histidine decarboxylase (HDC) in HMC-1 cells (Kempuraj et al., 2006). In keeping with the broad spectrum of activity exhibited by flavonols, myricetin, with an additional hydroxyl substituent on the 2-phenyl ring relative to quercetin, was also shown to inhibit mast cell degranulation (Kempuraj et al., 2005). The isoflavone genistein inhibited degranulation of HCMCs challenged with anti-IgE. Additionally, it inhibited phosphorylation of cellular proteins such as ERK-1 and ERK-2 which are involved in the downstream signalling cascade of activated mast cells (Suzuki et al. 1997). Similarly, genistein also inhibited histamine release and protein tyrosine kinase activation in BMMCs stimulated with antigen (Kawakami et al. 1992). The biflavone ginkgetin demonstrated dual cyclooxygenase-2/5-lipooxygenase inhibitory activity and was shown to inhibit the production of eicosanoids in BMMCs stimulated with c-*kit* ligand (KL). Additionally, ginkgetin inhibited release of β-hexosaminidase from these cells stimulated with KL (Son et al., 2005).

9.1.1 Flavonoid derivatives

Epigallocatechin gallate (EGCG), a constituent of green tea and related to the flavonoid family, demonstrated anti-allergic activity in both *in vitro* and in *in vivo* models. (Li et al.,

2005, Inoue et al., 2010). One of the primary constituents of silymarin, a mixture of polyphenolic flavonoids isolated from milk thistle (*Silybum marianum*), silibinin, was shown to inhibit the release of histamine and proinflammatory cytokines from RPMCs in a dose-dependent manner. *In vivo*, silibinin inhibited the PCA reaction (Choi and Yan 2009c).

10 Coumarins

Several reports exist which describe the mast cell stabilising properties of coumarins, a group of compounds sharing a 2*H*-chromen-2-one core. Scopletin, isolated from several plant species, inhibited the production of proinflammatory cytokines from human mast cells following challenge with various stimuli. However, scopletin did not affect the release of histamine from HMC-1 cells (Moon et al., 2007). Interestingly, scaporone, the methylated analogue of scopletin, dose-dependently decreased histamine release from rat mast cells stimulated by anti-DNP IgE, and inhibited PCA in rats. Scaporone also reduced the expression and secretion of proinflammatory cytokines (Choi and Yan 2009a).

Artekeiskeanol A, isolated from *Artemisia keskeana* Miq., suppressed degranulation of RBL-2H3 cells and also suppressed the mRNA levels of proinflammatory cytokines and phosphorylation of signalling kinases (Hong et al., 2009). Selinidin, a couramin from *Angelica keiskei*, attenuated the release of β-hexosaminidase from bone marrow-derived mast cells stimulated by antigen, reduced both the production of proinflammatory mediators and phosphorylation of PLCγ-1 and p38 MAPK (Kishiro et al., 2008). The furanocoumarin, 5-methoxy-8-(2-hydroxy-3-butoxy-3-methylbutyloxy)-psoralen, from *Angelica dahurica* inhibited both cyclooxygenase-2 and 5-lipoxygenase activity and generation of the lipid mediators, while also preventing degranulation of BMMCs activated with c-*kit* ligand (Hua et al., 2008). Interestingly, cinnamic acid, a precursor to the coumarin structure, markedly suppressed antigen-stimulated degranulation of β-hexosaminidase from RBL-2H3 cells through inactivation of Syk and PLCγ pathways (Ninomiya et al. 2010). Thunberginols A and B are isocoumarin derivatives which have shown anti-allergic activity by inhibiting histamine release from RPMCs *in vitro* (Matsuda et al. 2008). They both also inhibited degranulation of RBL-2H3 cells and the release of cytokines TNF-α and IL-4 (Wang et al., 2007). Thunberginol B was also shown to inhibit mRNA expression of several cytokines in RBL-2H3 cells stimulated by antigen (Matsuda et al., 2008). Rottlerin (2E)-1-[6-(3-Acetyl-2,4,6-trihydroxy-5-methylbenzyl)-5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl]-3-phenyl-2-propen-1-one, which also shares the chromene architecture was shown to inhibit IgE-induced

PLC $\gamma1$ and Akt phosphorylation, production of IP3 and increase in cytosolic Ca2$^+$ level in mast cells. It also prevented IgE-mediated cutaneous vascular extravasation, hypothermia, increase in plasma histamine level and tracheal tissue mast cell degranulation in a murine model dose-dependently (Chan et al., 2013).

11 Phenols

Magnolol and honokiol are phenolic structural isomers, isolated from the bark of *Magnolia obovata* that have shown to potently inhibit the degranulation of RBL-2H3 cells induced by IgE-antigen complex as well as the production of cytokines; IL-4 and TNF-α. Moreover, both compounds potently inhibited PCA reactions in mice (Han et al., 2007). Resveratrol, a phytoalexin, stilbene polyphenolic compound found in grapes, berries and peanuts, suppressed the expression of inflammatory cytokines such as TNF-α, IL-6 and IL-8 in PMACI induced- HMC-1 cells and decreased levels of intracellular Ca^{2+} (Kang et al., 2009). Hydroxytyrosol and its ester derivative, oleuropein inhibited concanavalin A, calcium ionophore A23187 and compound 48/80 induced degranulation when employed at a relatively high concentration of 100 μM (Persia et al., 2014). Likewise, the anti-allergic activity of polydatin, a resveratrol glucoside, showed similar properties (El-Agamy, 2012, Yang et al., 2012). Curcumin, a polyphenolic compound of *Curcuma longa* and related species, has demonstrated anti-allergic activity in both *in vitro* and *in vivo* models. It significantly inhibited antigen-induced degranulation and suppressed PCA reaction. Curcumin significantly inhibited the expression of mRNA for cytokines; IL-4 and TNF-α in a dose-dependent manner as well as their secretion in antigen-stimulated RBL-2H3 cells (Lee et al., 2008). The xanthones; mangostin-α, -β and -γ isolated from the pericap of *Garcinia mangostana* L. inhibited the release of histamine from IgE-sensitised RBL-2H3 cells in response to antigen through suppression of the signalling transduction pathway involving Syk and PLCγ (Itoh et al., 2008). Ellagic acid, a polyphenolic compound found in fruits and nuts such as raspberries, strawberries, walnuts and pomegranate has been shown to attenuate anti-IgE mediated allergic response *in vitro* and *in vivo*. Ellagic acid dose-dependently inhibited histamine release as well as the secretion of proinflammatory cytokines such as TNF-α and IL-6 from anti-DNP IgE induced-RPMCs. Ellagic acid attenuated anti-DNP IgE-mediated PCA in rats (Choi and Yan, 2009b). Hypothemycin, is a polyketide derived resorcylic acid lactone. It was shown block Kit activation, inhibit degranulation of both human mast cells

(HuMCs) and BMMCs as well as cytokine production at 10 µM. *In vivo*, hypothemycin reduced PCA reaction in mice (500 µg/30 g) (Jensen et al., 2008).

Emodin, an anthraquinone of Frangula bark and roots of *Reynoutria japonica Hout*, has been postulated to exert anti-allergic effects via inhibition of Syk phosphorylation in the proximal step reaction from the FcεRI as well as the phosphorylation of PKC–IKK2–SNARE complex in the late-stage pathway.

Within the flavonoid, coumarin and phenolic classes, it is evident from studies conducted to date that the precise mechanism by which these substances stabilise mast cells remains largely unknown. As with most planar molecules, as in these series, it is perhaps conceivable that many processes in the allergic cascade are targeted. This point is reinforced by the broad spectrum of biological actions exhibited by these classes of compounds and from the fact they show effects against many different cell populations. The hypothesis that truly selective inhibitors of a given molecular target can only be generated from three dimensional molecules is perhaps reinforced by findings within these studies. Nevertheless, as the allergic cascade may involve several inflammatory pathways, the idea of designing anti-allergic substances with multiple mechanisms of actions may have potential advantages therapeutically.

12   Terpenoids

Terpenes, a group of natural products characterised by repeating C5 units, have also displayed anti-allergic actions. A monoterpene/sesquiterpene (C10/C15) extract including borneol, camphene and linalool (C10) and nerolidol (C15) from *Amomum xanthiodes* showed good anti-allergic activity both *in vitro* and *in vivo*. (Kim et al., 2007). Sesquiterpene lactones displaying anti-allergic actions include parthenolide (Murphy et al., 1988, Miyata et al. 2008); dehydroleucodine, xanthatin (Penissi et al., 2009) and magnolialide (Lee et al., 2013). Nine types of sesquiterpene lactones from *Eupatorium chinense* L. suppressed the degranulation from antigen-stimulated RBL-2H3 and potently inhibited the PCA reaction (Itoh et al., 2009).

13   Nitrogen compounds

Alkaloids of several classes have displayed anti-allergic activity, either *in vitro* or *in vivo*. Structurally diverse examples include the morphinan sinomenine, which inhibits degranulation and cytokine production (Huang et al. 2008) and the indolinone indoline,

which interrupts granule exocytosis (Ruster et al., 2004; Kiefer et al., 2010). The marine alkaloid xestospongin C, an oxaquinolizidine, exerts its effects via blockade of IP$_3$ receptors on the endoplasmic reticulum membrane (Oka et al., 2002). Theanine, an amino acid present in green tea, inhibited histamine release from mast cells in a dose-dependent manner, suppressed the secretion of proinflammatory cytokines, and inhibited the PCA reaction *in vivo*. It is suggested that it acts as a mast cell stabiliser by preventing perturbation of the lipid bilayer of mast cells (Kim et al., 2012).

14   Semi-synthetic inhibitors of mast cell degranulation

Arguably, Nature has provided us with the greatest resource from which to develop novel mast cell stabilising agents. This is particularly exemplified by the promising activity exhibited by Khellin from *Amni visnaga*, which subsequently led to the development of DSCG. Our efforts and that of others stemmed from using nature's reservoir of relatively simple molecules to design synthetic derivatives with enhanced activity over that of their naturally derived precursors. In particular, the promising activity stemming from initial studies on the pterosin family of compounds, in particular, pterosin Z (Farrell et al 1996) led to the fruitful discovery of multiple families of benzocycloalkanol dimers arising from a series of manipulations to combinations of indanone (Sheridan et al. 2009a), tetralone and benzosuberone (Barlow and Walsh 2008, Barlow and Walsh 2010 and Barlow et al. 2011a and Barlow et al. 2011b) derived building blocks. These dimer based compounds inevitably required that the initial steps in their preparation revolved around a directed aldol condensation reaction to furnish the dimer, followed by a based catalysed alkylation reaction and carbonyl reduction to afford the alcohol series of dimers (Sheridan et al. 2009a). A second series involved coupling of the 3-bromo-indanone to either 1,2-aminoindanone or tetralin derivatives. Most of these studies employed compound 48/80 to induce histamine release from RPMCs, while selected examples also employed calcium ionophore A23187, concanavalin A as secretagous in the RPMC assay. The most potent analogues within these series were evaluated *in vivo* using the PCA assay and showed promising activity (Byrne et al*.,* 2011, Barlow and Walsh 2008, Barlow and Walsh 2010, Barlow et al. 2011a).

Recent work on photoswitchable chromone dimers, based on DSCG where the non-chromone part of DSCG was replaced by azo/azobenzene spacer groups revealed some interesting preliminary data on the three compounds tested as in the effect on activity of changing their cis:trans ratio following photisomerisation (Velema et al., 2013). The mast stabilising data

generated was sparse stemming from the low number of compounds evaluated, the use of only one cell line (LAD2) and only using compound 48/80 as elicitor.

15 Biologics

The most significant activating receptor expressed on mast cells is FcεRI. As expression levels of this receptor correlates with serum IgE levels, targeting serum IgE with specific anti-IgE antibody should result in the immobilisation of IgE and the subsequent reduction of free serum IgE which resulting in decreased expression of and load upon FcεRI (Belliveau 2005). Omalizumab (Xolair) is a humanised monoclonal antibody manufactured by recombinant DNA technology in the Chinese hamster ovary (CHO) cell line and is used in the treatment of asthma. Omalizumab selectively binds to the Cε3 domain of soluble IgE (Dodig et al., 2005) thus immobilising and preventing IgE-FcεRI binding. Clearance of the α-IgE-IgE complex is carried out by leukocytes and platelets. Mast cell FcεRI expression is downregulated after treatment and omalizumab shows effectiveness after at least 12-16 weeks. The decrease in serum IgE levels upon treatment with omalizumab occurs in a dose-dependent manner and treatment results in the inhibition of early and late phase allergic responses in asthma (Belliveau, 2005).

The complement-derived peptide C3a has also been evaluated as a possible inhibitor of mast cell degranulation and shown to inhibit degranulation of RBL-2H3 cells and BMMCs stimulated by antigen in a dose-dependent manner. It does so by interacting with the β-chain of FcεRI on mast cells which results in a decrease in the proximity of IgE binding to FcεRI and as a result suppresses the activating phosphorylation of tyrosine kinases and the activity of PLCγ (Erdei et al., 1999). A complement peptide derived from C3a, namely C3a9 was also shown to also prevent the immediate phase response of antigen stimulated-RBL-2H3 cells by triggering dissociation of tyrosine kinases, Lyn and Fyn with FcεRI and subsequent inactivation of downstream MAPK, p38 and extracellular-signal-regulated-kinase (ERK). This peptide also inhibited the late phase response of stimulated BMMCs by suppressing the secretion of proinflammatory cytokines such as IL-6 and TNF-α (Peterfy et al., 2008). Inhibition of mast cell degranulation by targeting inhibitory surface receptors has yielded promising results. Based on data generated to date, the inhibitory receptors, CD300a (Bachelet et al., 2006), Allergin-1 (Hitomi et al., 2010), LMIR3 (Izawa et al., 2012), FcγRIIB (Cemerski et al., 2012) and Siglec-8 (Kiwamoto et al., 2012) represent interesting targets for therapeutic intervention.

16 Conclusions

Mast cells continue to remain viable targets for natural product/synthetic chemists and molecular biologists alike stemming largely from the ever expanding role of this unique cell in both physiological and pathological conditions. Since the discovery of DSCG, significant progress has been made in the development of targeted therapies for receptors/enzymes expressed on mast cells. However, these targets are often not unique to the mast cell and as a consequence due consideration needs to be given to off target actions of new drug candidates undergoing clinical development. Interesting data continues to be generated on natural products as mast cell stabilisers, although the multi-modal action of some of these cast doubt on their precise mode of action. Indeed, as in the flavonoid class of natural products, several mechanisms are likely to be operating in tandem to explain their observed effect at the cellular or indeed *in vivo* level. As an example, there are over 11,500 papers cited in PubMed on the flavonoid quercetin with publications relating to its anti-cancer, anti-inflammatory, anti-allergy, anti-oxidant and anti-diabetic activity to demonstrate some of the diverse cellular targets of this planar molecule with appropriately placed phenolic groups. A significant number of the tyrosine kinase inhibitors in clinical use for the treatment of different types of cancer and as anti-angiogenics have also shown mast cell stabilising properties. While this is encouraging, it should be noted that the role of the mast cell in cancer progression is controversial (Theoharides and Conti, 2004). In some cases mast mediators are pro-angiogenic (for example heparin, interleukin-8 and vascular endothelial cell growth factor), while others are known to inhibit tumour growth (for example cytokines, such as IL-1, IL-4, IL-6 and tumor necrosis factor-α).

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