Current Options in the Treatment of Mast Cell Mediator-Related Symptoms in Mastocytosis

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Abstract: Patients with mastocytosis have symptoms related to the tissue response to the release of mediators from mast cells (MC), local mast cell burden or associated non-mast cell hematological disorders. MC contain an array of biologically active mediators in their granules, which are preformed and stored. MC are also able to produce newly generated membrane-derived lipid mediators and are a source of multifunctional cytokines. Mediator-related symptoms can include pruritus, flushing, syncope, gastric distress, nausea and vomiting, diarrhea, bone pain and neuropsychiatric disturbances; these symptoms are variably controlled by adequate medications.

Management of patients within all categories of mastocytosis includes: a) a careful counseling of patients (parents in pediatric cases) and care providers, b) avoidance of factors triggering acute mediator release, c) treatment of acute and chronic MC-mediator symptoms and, if indicated, d) an attempt for cyto reduction and treatment of organ infiltration by mast cells.

Keywords: Mastocytosis, mast cell-mediator related symptoms, treatment.

INTRODUCTION

Mastocytosis comprises a group of rare disorders with diverse clinical presentations, characterized by an abnormal increase in tissue mast cells (MC) including skin, bone marrow, bone, gastrointestinal tract, liver, spleen, and lymph nodes [1]. As in other orphan diseases, health care professionals have limited experience with its diagnosis, classification and management.

Mastocytosis is classified into 7 categories based on clinical presentation and the pattern and extent of tissue involvement. Cutaneous mastocytosis (CM) is limited to the skin and characterized by increased numbers of subepidermal and dermal accumulations of mature tryptase and chymase positive MCs, frequently in perivascular locations [2]. Systemic mastocytosis (SM) defines multiple entities in which MCs infiltrate bone marrow and internal organs with or without skin involvement [3]. Indolent systemic mastocytosis (ISM) is the most common form, and is associated with a good prognosis and a normal life expectancy in a high percentage of patients. Aggressive mastocytosis (ASM), SM associated with a clonal hematological non-MC lineage disease (SM-AHNMD), mast cell leukemia (MCL) and mast cell sarcoma (MCS) are uncommon forms of mastocytosis, and are associated with a poor prognosis. Extracutaneous mastocytosis is a rare variant with benign morphological features.

The diagnosis of systemic mastocytosis (SM) is based on major and minor criteria as established by the 2000 Vienna Working Conference on Mastocytosis. Major criteria include the presence of multifocal infiltrates of more than 15 MCs per high-powered field in sections of bone marrow or other extracutaneous tissues, and minor criteria include the presence of 25% spindle-shaped abnormal MCs in aspirates of those infiltrates, the expression of CD25 (with or without CD2) on CD117 (Kit) positive MCs, the presence of c-kit somatic mutations and the elevation of serum total tryptase levels >20ng/ml [3].

Patients with CM and SM may present symptoms related to the local MC infiltration and to the tissue response to mediators released from MCs. The age of onset of the disease (pediatric or adult), number of tissues involved, and clinical behavior (indolent or aggressive) provide further heterogeneity.

A rational management approach in mastocytosis must include: a) a careful counseling of patients (parents in pediatric cases) and care providers, b) avoidance of factors triggering acute mediator release, c) symptomatic treatment of acute and chronic mast cell-mediator release, and, if indicated d) reduction of the local or systemic mast cell burden. Cytoreductive therapy has been used in aggressive forms of the disease such as ASM, MCL/MCS, as well SM-AHNMD. Among these, interferon alpha (IFN-α) [4, 5], the purine analog chlorodeoxyiadenosine (2-CDA, cladribine™) [6], and the tyrosine kinase inhibitor imatinib mesylate (Gleevec™, Glivec™) for patients lacking codon 816 c-kit mutations [7], are most relevant. However, few patients suffering from mastocytosis should need cytoreductive therapy given the associated complications. Pediatric cases and ~85% of those with adult mastocytosis are not candidates for cytoreductive therapies.

The goal of this review is to provide an overview of the symptoms of mastocytosis, which can be encountered in all
disease categories, and the current therapeutic options for their management.

**MAST CELL MEDIATOR-RELATED SYMPTOMS**

Symptoms of mastocytosis include flushing, pruritus, urtication which can be associated to systemic complaints of abdominal pain, nausea, vomiting, diarrhea, bone pain, headaches or vascular collapse. Symptoms derive from multi-organ MC involvement with prominent skin, gastrointestinal, bone, vascular and neuropsychiatric complaints. Linking symptoms to MC-derived mediators depend on the known actions of the mediators and the efficacy of target based interventions, which suppress or control those symptoms [8].

**General and Constitutional Symptoms**

Constitutional symptoms such as fatigue, weight loss, fever and sweats occur in long-standing ISM and can be the presenting symptoms for the aggressive forms including ASM, SM-AHNMD and MCL [9]. MC cytokines such as TNFα, IL-1β and IL-6 can contribute to the constitutional symptoms [10], and their release has been measured in mastocytosis patients.

**Cutaneous Signs and Symptoms**

Typical CM lesions include urticaria pigmentosa (UP), nodular mastocytomas, diffuse cutaneous mastocytosis (DCM) and telangiectasia macularis eruptiva perstans. The spontaneous or induced release of MC mediators in the skin produces flushing, pruritus, urticaria and dermatographism [2] in patients with CM and SM.


Cutaneous mastocytosis of infancy and childhood is a benign disease; most solitary mastocytomas in children involute spontaneously by age 10 years or earlier, while UP and DCM regress at puberty in many cases. Activating (codon 815 and 816) and non-activating (codon 839) mutations of c-kit receptor expressed on cutaneous MC have been identified in children with UP, which may persist after clinical resolution [21].

Persistent CM of childhood and adulthood can lead to SM [22]. However, regression of UP in adult patients with ISM may or may not be accompanied by systemic disease improvement [23].

High doses of aspirin which block prostaglandin generation, are proven effective at inhibiting flushing in some patients who are able to tolerate this drug, indicating that PGD2 is involved [24]. The combination of H1 and H2 anti-histamines has been effective in pruritus and preventing urticaria, indicating that histamine is also involved [9]. Psoralen plus ultraviolet A (PUVA) photochemotherapy decreases skin MC numbers, fading UP lesions in adults and decreasing the formation of bullae in children [25] (see treatment of diffuse cutaneous mastocytosis).

**Gastrointestinal Symptoms**

Gastrointestinal symptoms can be chronic and severe and represent a leading source of complaints in patients with mastocytosis. Abdominal pain and diarrhea are more frequent than nausea and vomiting. Gastrointestinal pain can be of typical dyspeptic nature representing peptic ulcer or gastroesophageal reflux disease as a consequence of MC mediator release, but acid hypersecretion is not a consistent finding [26]. The gastric parietal MC mass is normal in most patients with ISM as well as maximal acid output in response to pentagastrin with normal gastrin, VIP, motilin or substance P levels [27]. Non-dyspeptic and crampy abdominal pain can be triggered by alcohol, stress and hot and spicy foods. Small intestine involvement leading to malabsorption and mild steatorrhea has been described [28].

Anti-histamine H1 and H2 receptor blockers reduce acid hypersecretion and control diarrhea [9], implicating histamine. Oral sodium cromolyn is minimally absorbed, but its ability to control abdominal pain and diarrhea is attributed to stabilization of intestinal MCs [29].

Patients with long-standing ISM can present with hepatomegaly with normal liver function and minimal elevation of liver enzymes [9]. Portal hypertension can develop in rare cases with transudative ascites, due to intrahepatic venous obstruction [28].

**Bone Marrow and Lymphatic Tissues**

Patients with SM present increases of bone marrow MCs with atypical morphologies [30]. By flow cytometry, pathological MCs show aberrant expression of CD25 and CD2 antigens and abnormally high levels of the CD35 complement receptor, the CD59 complement regulatory molecule, and the CD69 early-activation antigen [31,32]. The presence of dense compact aggregates of spindle shaped MCs in bone marrow biopsies, stained with immunohistochemical techniques using antibodies against tryptase is diagnostic [33]. In MCL, there is an extensive infiltration of atypical and immature MCs replacing the normal marrow architecture [30]. Bone marrow fibrosis with decrease in other hematopoietic lineages and extramedullary hematopoiesis can be seen in long-standing SM or aggressive mastocytosis (ASM) [34].

Splenomegaly is frequent with fibrosis and thickened capsule due to MC infiltration in association with eosinophils [35] Lymphadenopathy occurs in aggressive forms [35] or in long-standing ISM with focal or diffuse MC infiltration. Immature poorly metachromatic MCs expressing c-kit and SCF were found in the spleen and lymph nodes of a patient with ASM [34].

Prominent fibrotic changes are present in the bone marrow, liver, spleen and lymph nodes of some patients with ISM and ASM, implicating IL-13 and TGFβ as the MC mediators.

**Skeletal Symptoms**

Bone pain is a frequent complaint involving long bones (femur, pelvic bones), but small bones (skull, ribs, spine, hands) and joints can also be affected [36]. Bone scan
evaluation reveals a mixture of lesions ranging from osteopenic, lytic or sclerotic changes often misinterpreted as osteoporosis, metastatic disease, Paget’s disease of the bones or multiple myeloma [9]. Most reports on the presence of osteopenia in mastocytosis include a low number of cases [10,37-39] and prospective studies on the prevalence of osteopenia in mastocytosis have not been published. Those changes, which can lead to vertebral compression fractures in some cases [40], may not be explained only with the extent of MC infiltration of the bones but possibly by mast cell derived soluble factors interfering with normal bone metabolism. IL-1b, IL-6 and tumor necrosis factor secreted by MCs can mediate bone remodeling [41-46].

Cardiovascular Symptoms

Death has been reported due to severe and prolonged cardiovascular collapse in patients with SM. Spontaneous or triggered syncope and vascular collapse are dramatic acute manifestations of SM [9,28]. Patients often describe typical prodromal symptoms such as lightheadedness, palpitations and tachycardia before the syncopal episode and retrograde amnesia after the episode.

Histamine and prostaglandin PGD2 have been implicated as the mediators of acute vascular collapse and hypotension [47,47,48].

Neuropsychiatric Symptoms

A mixed organic brain syndrome has been described in patients presenting chronic neuropsychiatric symptoms. Those patients are often misdiagnosed due to the non-specific nature of the symptoms and the lack of studies of MC activity in the human brain in patients with SM. Symptoms include decreased attention span, difficulty in concentration, forgetfulness, irritability, depression, poor motivation, confusion, anger, anxiety, lethargy and sleepiness [49]. Headache is a frequent complaint with heterogeneous presentation. It can be dull, vascular migraine-like or histaminergic with rhinorhea, pruritus and lacrimation [9].

PGD2 has been found as a critical mediator of sleep/awake patterns in transgenic mice and rats [50,51], and blockade of its secretion has improved neuropsychiatric symptoms in patients with SM. Cromolyn sodium has been reported to reduce the symptoms of the mixed organic brain syndrome [52].

Pulmonary Symptoms

Pulmonary symptoms are not a prominent feature of mediator-related symptoms in patients with mastocytosis, and wheezing and bronchial obstruction have only been reported during spontaneous or triggered massive MC mediator release. Post mortem findings do not support an increase in pulmonary MCs, indicating that homing of MCs to the lung is limited in mastocytosis patients [28].

Pregnancy

Successful pregnancies have been reported in patients with SM. Infertility rates have not been reported to be different than the general population. Mild exacerbation of MC-mediator release has complicated pregnancy and vaginal deliveries in few women. No deaths have been reported during delivery [53].

PHARMACOLOGICAL BASIS OF THERAPEUTICS

Antihistamines

Histamine

Histamine (ß-imidazolylethylamine) is the only known biogenic amine produced by human mast cells and basophils, and is formed from histidine by histidine decarboxylase. Most of this histamine is then stored in secretory granules, to be released from activated cells by degranulation; although a portion may be released constitutively. Histamine is the only preformed mediator of human mast cells with direct potent vasoactive and smooth muscle spasmodic effects. Histamine diffuses rapidly from its sites of release, but is normally metabolized within minutes of release, suggesting that it is destined to act quickly and locally. Human mast cells contain 1 to 3 pg of histamine per cell. Histamine concentrations of about 0.1 M are estimated to exist inside secretory granules; whereas concentrations of about 2 nM exist in plasma. Histamine levels in samples obtained from sites outside of the circulation reflect local rates of production and removal. In subjects with systemic mastocytosis, elevated levels in plasma occur during intervals without acute symptoms of immediate hypersensitivity, and to a greater magnitude during anaphylaxis.

Histamine exerts its biological and pathobiological effects through its interaction with cell specific receptors designated H1R, H2R, H3R, and a newly identified H4R [54], which initially were defined with the recognition of specific agonists and antagonists. H1R [55] and H2R [56] each have seven regions predicted to span the plasma membrane, and are G protein-coupled receptors. H1Rs are blocked by chlorpheniramine; H2Rs are blocked by cimetidine; and H3Rs and H4Rs are blocked by thioperamide. Examples of receptor specific agonists include 2-methylhistamine at H1Rs, dimaprit at H2Rs, α-methylhistamine at H3Rs, and clobenpropit at H4Rs.

Effects of histamine mediated by H1Rs include enhanced permeability of postcapillary venules, vasodilation, contraction of bronchial and gastrointestinal smooth muscle, and increased mucus secretion at mucosal sites. Increased vasopermeability will facilitate the tissue deposition of factors from plasma that may be important for tissue growth and repair and of foreign material or immune complexes that result in tissue inflammation. Histamine by an H1R-dependent mechanism in rodents also activates endothelial cells to transfer P-selectin from internal Weibel-Palade bodies to the cell surface, thereby enhancing neutrophil rolling and recruitment [57,58]. H1R-/- mice exhibit modest neurological alterations, but show no apparent developmental abnormalities [59]. Studies on mice have demonstrated that histamine enhances Th1 T cell function through H1R pathway; while H1R-/- mice exhibit reduced Th1 responses and increased IgE production [60]. Whether the chronically elevated histamine levels of mastocytosis...
subjects enhance Th1 over Th2 responses, thereby reducing atopy, needs to be considered. Of greater certainty is the increased risk of peptic ulcer disease in patients with systemic mastocytosis due to histamine-mediated gastric acid production in the stomach, a problem that can be largely prevented by prophylactic administration of H2R antagonists.

The combined effects of H1 receptor and H2 receptor-mediated activities of histamine are required for the full expression of vasoactivity. For example, the "triple response" caused by an intradermal injection of histamine, namely a central erythema within seconds (histamine arteriolar vasodilation), followed by circumferential erythema (axon reflex vasodilation mediated by neuropeptides) and a central wheal (histamine vasopermeability, edema) peaking at about 15 minutes, is mostly blocked by H1 receptor antagonists, but is completely blocked only with a combination of H1 and H2 receptor antagonists [61]. Analogous results have been observed for the tachycardia, widened pulse pressure, diastolic hypotension, flushing and headaches resulting from intravenous infusion of histamine [62]. Importantly, although antihistamines block the engagement of histamine receptors by histamine, once stimulation of cells has been initiated through these receptors \textit{in vivo}, there is no evidence that administration of antihistamines will substantially alter the clinical response. Thus, prophylactic administration of antihistamines is far more effective than their rescue administration at reducing the histamine-mediated component of anaphylaxis or of chronically elevated levels of histamine in the circulation and tissues.

1/ H1-Receptor Antagonists

H1R antagonists can be considered in the context of their sedating properties, which relate to how well they cross the blood brain barrier. Fexofenadine is non-sedating at both approved and off-label higher doses. Cetirizine, desloratidine and loratidine are non-sedating in most subjects at standard doses, but become sedating at higher doses. Older antihistamines, such as hydroxyzine, diphenhydramine, brompheniramine, ketotifen and chlorpheniramine, provoke feelings of drowsiness in a substantial portion of subjects, and impair functional responses and sleep patterns in nearly everyone [63]. Consequently, those who use sedating H1R antagonists must be cautioned to not drive or perform other activities in which inattention would be dangerous to themselves or others. Doxepin has both H1R and H2R blocking ability as well as being a mild antidepressant, and may be useful in some mastocytosis patients with neuropsychiatric manifestations.

2/ H2 Receptor Antagonists

H2 receptors are widely distributed in tissues and cells such as gastric parietal cells, vascular smooth muscle [64-69], suppressor T cells [70], brain [71], heart [72-74] among others (reviewed in reference [75]). Histamine H2-receptors have a potent effect on gastric acid secretion, and the inhibition of this secretory process by H2-receptor antagonists has provided evidence for an important physiological role of histamine in the regulation of gastric secretion [68,76]. H2 receptors also participate in the control of vascular permeability (reviewed in reference [75]).

A large number of compounds with H2-receptor antagonist properties have been developed; cimetidine, famotidine and ranitidine being the most relevant. Histamine H2 antagonists can decrease abdominal symptoms in patients with systemic mastocytosis [9,27,77-81] mainly dyspeptic pain related to gastric acid hypersecretion. It has been reported that standard doses of H2 antagonists may not adequately inhibit acid secretion [26], similar to what has been described in other hypersecretory disorders [82] and, thus, higher or more frequent doses may be necessary [26]. Patients with dyspeptic pain refractory to H2 antagonists could obtain additional benefits with the simultaneous use of a proton pump inhibitors.

Tryptase

Tryptase (EC3.4.21.59) is normally expressed by both human mast cells and basophils, and is derived principally from two genes that are tandemly arranged on chromosome 16p13.3 such that there is either one \( \alpha \)-tryptase gene and one \( \beta \)-tryptase gene, or two \( \beta \)-tryptase genes per haploid chromosome [83]. Approximately 25% of people are \( \alpha \)-tryptase-deficient [84-86]. Both \( \alpha \)-tryptase and \( \beta \)-tryptase mRNAs are expressed in all types of human mast cells [87,88]. Blasts from certain subjects with acute myelocytic leukaemia [89] over-express primarily \( \alpha \)-tryptase mRNA and protein.

Heparin facilitates autoprocessing of \( \beta \)-protryptase to \( \beta \)-pro’tryptase, pro’ to mature \( \beta \)-tryptase, and conversion of mature \( \beta \)-tryptase monomers to tetramers [90], and also stabilizes the tetramer by binding to a cationic groove that spans each dimer of the tetramer [91,92]. Mature \( \beta \)-tryptase, the principal enzymatically active form of tryptase, is stored in the secretory granules of human mast cells [93] in a complex with proteoglycan, presumably heparin [91-96]. The solution to the crystal structure of \( \beta \)-tryptase [91] showed that all active sites face into the small, central pore of the planar tetramer, thereby restricting access to these sites by inhibitors and substrates.

Without stabilization by a polyanion active tetramers spontaneously convert to inactive monomers, more readily at neutral than acidic pH [96]. Conversion of monomers back to tetramers is facilitated by heparin, but occurs only at acidic pH. The acidic pH that nurtures tryptase processing and reactivation of monomers also may regulate enzymatic activity. \( \beta \)-tryptase degrades fibrinogen ~50-fold faster at pH 6 than at 7.4 [97]. Release of \( \beta \)-tryptase at sites of acidic pH (airway mucosal surface, foci of inflammation and areas of poor vascularity, e.g. solid tumor margins and wound healing sites), might be optimal for the enzyme, while diffusion away from such sites would result in reduced proteolytic activity. Such a mechanism would tend to limit the activity of \( \beta \)-tryptase to its local tissue site of release.

Although heparin-stabilized tetramers are enzymatically active at both acidic and neutral pH, heparin-stabilized monomers are only active at acidic pH. Biologic protease inhibitors such as antithrombin III (ATIII) and \( \alpha \)-2-macroglobulin (\( \alpha \)2M), prevent reactivation of inactive
monomers to active tetramers by inhibiting the active monomeric intermediate [98]. Mast cells also produce a protease inhibitor, SerpinB6, that can form complexes with trypstat [99]. In contrast, α-trypstat may not undergo autoprocessing from protrypstat to pro’trypstat, because a trypstat-resistant Q^3 rather than a trypstat-sensitive R^3 is present in the -3 position of the propeptide [83,90]. Also, when mature recombinant α-trypstat is produced in vitro, though it forms a tetramer, the protein appears to be enzymatically inactive [100-102].

Two immunoassays, one recognizing precursor and mature forms of α-trypstat and β-trypstat (total trypstat), and another recognizing only mature trypstat are utilized to assess these different forms of trypstat. Precursor forms (pro/pro’^) of both α and β trypstat are secreted spontaneously by unstimulated cells, while mature β-trypstat is released from activated mast cells and basophils by degranulation [86]. In lung- and skin-derived mast cells, mean tryptase levels of 11 and 35 pg/mast cell, respectively, account for a substantial portion of the cell protein [103]. In contrast, mean levels in peripheral blood basophils (0.05 and 0.04 pg/basophil) [104-106] are typically less than 1% of those in mast cells, even though histamine levels per cell are similar. Although α-trypstat levels are undetectable in normal serum (<1 ng/ml), they are elevated in the blood of most cases of systemic anaphylaxis with hemodynamic compromise, particularly when the precipitating agent is administered parenterally. In insect sting-induced anaphylaxis, the magnitude of mast cell degranulation appears to be the primary determinant of clinical severity [107-109]. In contrast, the mean total tryptase level in baseline serum from healthy individuals is 4.9 ± 2.3 ng/ml (mean ± SD) [110]. Total tryptase levels are elevated in most subjects with systemic mastocytosis (≥20 ng/ml), and reflect the total body burden of mast cells [111]. Further, ratios of total to mature trypstat are typically ≥20 in systemic mastocytosis during baseline intervals and <10 during systemic anaphylaxis in subjects with a normal mast cell burden. The trypstat genotype has a modest affect on total serum tryptase levels in healthy subjects; each α-trypstat gene is associated with a modest (~0.4 ng/ml) increase. Preliminary data among mastocytosis patients has not detected a significant effect of the trypstat genotype on serum levels of total trypstat.

The biologic activity(ies) of enzymatically-active trypstat are not obvious from the involvement of mast cells in atopic diseases. The most relevant biologic substrate(s) of trypstat remain uncertain, though many potential ones have been evaluated, primarily in vitro. Predicted biologic outcomes might include anticoagulation, fibrosis and fibrolysis, kinin generation and destruction, cell surface PAR-2 activation, enhancement of vasopermeability, angiogenesis, inflammation, and airway smooth-muscle hyperreactivity. Showing the importance of these potential activities in vivo remains a challenge. The emerging availability of pharmacologic inhibitors of trypstat, and preliminary studies in animals suggesting they attenuate the bronchial response to an allergen challenge may facilitate identification of the most important biologic substrates [112,113].

**Chymase**

Chymase, located on human chromosome 14, is one of two principal enzymes accounting for the chymotrypsin-like activity present in the human cutaneous mast cells [114,115]. Chymase was selectively localized to a subpopulation of mast cells [116,117], which also is the phenotype of mast cells in urticaria pigmentosa lesions. Dispersed skin-derived mast cells contain ~4.5 pg of chymase/mast cell. Human chymase is a monomer of 30,000 daltons [118]. Similar to trypstat, chymase is a serine esterase that is stored fully active in mast cell secretory granules, presumably bound to proteoglycan. Heparin facilitates processing of prochymase to active chymase [119], and either attracts or repulses potential chymase substrates based on ionic forces [120]. Unlike trypstat, chymase stability is not substantially affected by heparin and its activity is inhibited by classical biologic inhibitors of serine proteinases, such as α1-antichymotrypsin, α1-proteinase inhibitor, and α2-macroglobulin [121].

Potential biologic activities of chymase, like those of trypstat, are based on in vitro observations. Chymase is a potent activator of angiotensin I, inactivates bradykinin and PAR-1 receptors, stimulates mucus production and leukocyte recruitment, processes procollagen to collagen fibrils, cleaves Kit from the cell surface and attacks the lamina lucida of the basement membrane at the dermal-epidermal junction of human skin. As for trypstat, the activities of importance in vivo remain speculative.

**Carboxypeptidase A3**

Human mast cell carboxypeptidase A3, a zinc-dependent exopeptidase, resides with chymase and cathepsin G in secretory granules [122]. Stored fully active, at neutral pH it cleaves the carboxy terminal His^9^-Leu^10^ bond of angiotensin I. Human mast cells dispersed from skin contain 5 to 16 pg of carboxypeptidase A3 per cell. This enzyme is a monomer with a molecular weight of 34,500 [123]. Its substrate specificity is for carboxy terminal Pehe and Leu residues.

Concerted activity of these mast cell proteases could greatly alter the biologic activities revealed when each is analyzed by itself. An example of this principal has been reported for trypstat and elastase. Neither of these enzymes generates bradykinin from high molecular weight kininogen, but together they generate bradykinin from both oxidized and non-oxidized high molecular weight kininogen [124]. Whether chymase and trypstat act by this principal remain to be seen. Further, the cleavage by chymase next to aromatic amino acid residues would provide potential substrates for carboxypeptidase A3.

**Inhibition of Membrane Derived Lipid Mediators from Mast Cells**

1/ Prostaglandins: Aspirin, Non-Steroidal Anti-Inflammatory Drugs and New COX-2 Inhibitors

Prostaglandins are mast cell metabolic products of arachidonic acid (AA) derived from membrane phospholipids through the action of phospholipase enzymes.
PLA2 [125]. The release of AA occurs within minutes of IgE cross-linking of mast cell FcεRI receptors [126]. Aspirin-sensitive prostaglandin endo/cycloperoxide synthase 1 and 2 (COX-1 and COX-2) isoenzymes, catalyze the conversion of arachidonic acid to PGH2 [127] and PGD synthase convert PGH2 to PGD2. PGD2 has multiple functions, including the inhibition of platelet aggregation. In the lung, PGD2 induces bronchoconstriction upon inhalation and sensitized mice deficient in the PGD2 receptor DP, exhibit decreased airway responses to antigen and diminished eosinophil infiltration [128]. In the skin, PGD2 produces a wheal and flare response due to vasodilation and increased vasopermeability [129]. Flushing in response to niacin is mediated by PGD2 [130]. In the brain, transgenic mice overexpressing the PGD2 synthase gene and producing increased levels of of PGD2 have a significant increase in non-REM sleep and a dramatic decrease in locomotor activity after tail clipping compared to controls [51]. Furthermore, PGD2 is found to regulate sleep patterns in rats and cerebro-spinal fluid PGD2 concentration has a circadian rhythm with increased levels during sleep deprivation [50]. There is evidence for increased acute and chronic production of PGD2 and its major metabolite 9a, 11b-PGF2 in the urine and serum of patients with SM [48,131,132]. Because aspirin and NSAIDS effectively block the production of the PGD2 precursor PGH2, their use in SM patients has been recommended. No studies are available on the efficacy of NSAIDS on patients with SM but flushing is effectively blocked with doses of aspirin above 1g daily [133]. However, it should be noted that NSAIDS and aspirin may trigger mast cell mediator release and anaphylaxis in some patients, and should not be used in patients with known or suspected sensitivities to these drugs. New, specific COX-2 inhibitors may be a potential avenue to replace aspirin and NSAIDS in patients with SM, although their efficacy in this disease has not been explored.

2/ Leukotrienes: 5-LO Inhibitors and Leukotriene-Receptor Antagonists

Leukotrienes (LT), like prostaglandins, are mast cell metabolic products of arachidonic acid derived from membrane phospholipids through the action of phospholipase enzymes PLA2. Arachidonic acid released by PLA2 is processed by 5-lipoxygenase (5-LO) into hydroperoxy eicosatetraenoic acid (5-HPETE) and then to LTA4 [52]. The predominant LT in mast cells is the cysteinyl leukotriene LTC4, which is produced by LTC4 synthase through the conjugation of LTA4 to glutathione. LTC4 is converted into the active metabolites LTD4 and LTE4 by cleavage of glutamic acid and glycine [134].

Cysteinyl leukotrienes including LTC4 have vasoactive actions in multiple organs. After skin injection, they increase microvascular permeability and induce long lasting wheal and flare responses [129,135]. Upon inhalation, LTC4 and LTD4 induce bronchoconstriction in normals with a thousand fold greater potency than histamine [136]. Urinary LTE4 (N-acetyl LTE4) is found elevated in patients with aspirin intolerance at baseline and increases further after aspirin challenge [137]. Inhibitors of 5-LO block bronchoconstrictive responses to cold air and to aspirin challenge in asthmatic patients [138]. Cysteinyl leukotriene receptor antagonists selective for the type 1 receptor (montelukast) are effective at attenuating early and late responses after allergen and exercise challenges and are efficacious in the management of chronic bronchial asthma [139]. Although there are no reports on urinary leukotrienes in patients with SM, a recent report on the efficacy of montelukast in a child with bullous skin mastocytosis, hepatomegaly and wheezing, clearly implicates these mediators in SM [140]. The use of 5-LO inhibitors has been recommended in SM.

3/ PAF: Platelet Activating Factor

PAF is produced by acetylation at AA after PLA2 cleavage from membrane phospholipids and is found in platelets, eosinophils, neutrophils, mast cells, endothelial and epithelial cells. Human recombinant PAF induces shock and death when injected intravenously in mice [141] and its effect is inhibited by PAF acetylhydrolase [142]. Mice lacking the PAF receptor have a significant reduction in this response.

Inhaled PAF causes bronchoconstriction and increased bronchial responsiveness in normal subjects and in asthmatics. PAF is 1000 times more potent than histamine at inducing increased vasopermeability in humans. PAF has been found elevated in skin in diffuse cutaneous mastocytosis [143], but no PAF antagonist have been used in patients with SM.

Mast Cell Stabilizers

1/ Sodium Cromolyn

The mechanism of action of sodium cromolyn is poorly understood. In permeabilized mast cells, sodium cromolyn inhibits GTP-γ-S-induced secretion by a mechanism not involving NDPK [144]. In activated neutrophils sodium cromolyn selectively inhibited O2- generation but not degradation and such effect may be associated with inhibition of assembly of an active NADPH oxidase in the neutrophil and prevention of oxygen radical-induced tissue damage [145]. In a recent study, inhibition of MC-mediated immediate-type hypersensitivity by disodium cromoglycate has been reported [146].

Oral sodium cromolyn has been successfully used in the control of several mast cell-mediator related symptoms such as abdominal pain, diarrhea [29,52,147-155], mixed organic brain syndrome [49], to restore cognitive abilities [14], and in a lesser extent in other manifestation such as pruritus and flushing. Furthermore, improvement of bone pain has been described in a case of mastocytosis with bone sclerosis [156]. The recommended adult dosage is 200 mg four times /d and doses ranging from 60 to 100 mg have been used in children.

On the other hand, treatment with topical cromolyn cream in a hydrophilic emollient vehicle to a final concentration of 0.21% has a significant anti-inflammatory effect on moderate-to-severe atopic dermatitis [157] and beneficial effects have also been obtained in cutaneous manifestations of mastocytosis in children (L. Escribano, personal communication 1999).
Interferon-α is a naturally occurring cytokine with antiproliferative, immunomodulatory and antiviral effects. Four types of interferon-α preparations exist in the U.S.: IFN-α2a, IFN-α2b, IFN-α-1b, and IFN alfacon. IFN-α2a and IFN-α2b are produced in E. coli by recombinant DNA technology, and differ from each other by 1 amino acid at codon 23. IFN-α-1b is a mixture of IFN-α proteins obtained from pooled human leukocytes infected with Sendai virus. IFN alfacon is a non-naturally occurring protein synthesized in E. coli with a modified copy of the IFN-α gene.

IFN-α has been used in the treatment of chronic hepatitis C, as well as a variety of neoplastic and myeloproliferative disorders such as chronic myeloid leukemia, essential thrombocytopenia, polycythemia vera, idiopathic hypereosinophilic syndromes, hairy cell leukemia, AIDS-related Kaposi’s sarcoma, renal cell carcinoma, multiple myeloma and melanomas. The mechanism of action of IFN-α in myeloproliferative disorders is not well understood, although the wide spectrum of the lineages affected by its administration suggests that it acts at the level of a pluripotential hematopoietic progenitor cell.

IFN-α2b (1-3 million units, three times weekly) with or without oral glucocorticoids has been used in the treatment of systemic mastocytosis [4]. A recent review of the literature retrospectively applying newly proposed disease response criteria concluded that IFN-α had resulted in a major response (complete resolution of end organ dysfunction attributable to mast cell disease) in approximately 20% of the patients [173]. However, complete resolution of the bone marrow disease in response to IFN-α therapy is rare even when there is symptomatic and hematologic improvement [81,174]. A recent prospective multicenter phase II trial on 20 patients reported minor responses consisting of symptomatic improvement of flushing and abdominal complaints accompanied by small decreases in circulating mast cell mediator levels and attenuation of UP skin lesions in some patients, although the degree of bone marrow infiltration by mast cells was unchanged [175]. Another study on 5 patients with aggressive systemic mastocytosis reported a major response in 2, partial response in 1 and stable disease in 1 patient while 1 patient progressed to mast cell leukemia [176]. This study suggested consideration of the use of IFN-α2b with prednisone as a first line therapy in patients with aggressive systemic mastocytosis. IFN-α has also been used successfully in patients with mast cell disease associated osteopenia and osteoporosis where the use of the drug resulted in improvement of bone pain and bone mineralization [177,178].

Duration of therapy with IFN-α in mastocytosis is not established. Some authors suggest continuation of therapy as long as the clinical response persists and the drug is tolerated [173,176]. Most common side effects of IFN-α leading to discontinuation of therapy include flu-like symptoms, cytopenias and depression. The latter two conditions may result in life-threatening complications. In addition, serious cardiotoxicity is reported in some patients on IFN-α therapy [179]. It is therefore important to consider risk-benefit ratio prior to selection of patients with treatment with IFN-α. Based on available literature, this population appears to be patients with aggressive or myeloproliferative variants of mastocytosis or those with clinically significant osteoporosis unresponsive to therapy with bisphosphonates.

Kinase Inhibitors

Imatinib

Mastocytosis is associated with gain-of-function mutations of the c-kit protooncogene at codon 816 (most commonly D816V) [180,181]. C-kit gene encodes the Kit protein which is a transmembrane receptor with intrinsic tyrosine kinase activity. Dimerization of Kit by its ligand, stem cell factor (SCF), triggers autophosphorylation of tyrosine residues and creates docking sites on the molecule for further downstream signal transduction molecules, which ultimately lead to activation, proliferation, migration and differentiation of mast cells [181]. D816V point mutation, located within the enzymatic domain of kit, obviates the need for SCF binding for activation to occur [182]. Therefore, the receptor protein with codon 816 mutation is constitutively phosphorylated at its baseline. This autoactivation of the receptor is thought to be involved in neoplastic transformation of the mast cell, as c-kit carrying similar mutations, when introduced into factor dependent cell lines, resulted in factor independent growth of hematopoietic cell lines [183], and mice transgenic for the murine equivalent for the mutation developed hematologic neoplasms [184].
Development of small molecular weight inhibitors of tyrosine kinase thus generated much excitement about the possibility of a curative treatment of mastocytosis. Imatinib (Gleevec), the first of such small molecular weight inhibitors to be approved for human use, inhibits c-kit as well as bcr-abl and PDGF-R tyrosine kinases. Imatinib is currently approved for treatment of bcr-abl positive chronic myeloid leukemia and Kit-expressing gastrointestinal stromal tumors (GIST). The latter tumor is associated with gain-of-function mutations of c-kit at the juxtamembrane domain, which is located intracellularly between the transmembrane and tyrosine kinase domains of the molecule. Therapy with imatinib also produced dramatic responses in a patient carrying a novel activating mutation at the transmembrane c-kit domain, who showed a dramatic response to imatinib with clinical and histopathological improvement and a reduction of serum tryptase levels [190].

A careful analysis of a lesional tissue sample (such as bone marrow or skin) for codon 816 c-kit mutations is thus essential to predict response to imatinib. Considering the high incidence of codon 816 c-kit mutations in lesional mastocytosis tissues [191,192], most patients with adult onset systemic mastocytosis do not appear to be candidates for imatinib therapy.

TREATMENT OF MAST CELL MEDIATOR RELATED SYMPTOMS

1/ Avoidance of Factors Triggering Mast Cell Mediator Release

It is generally accepted that patients suffering from mastocytosis is at higher risk for severe mast cell-mediated release than the non-mastocytosis population; nevertheless, the exact percentage of patients at risk for severe mast cell-mediatore release related to different triggering factors is unknown. For this reason, avoidance of known and possible triggering factors constitutes a major goal in the management of the disease. Clinical evaluation of patients at diagnosis should include a careful clinical inquiry for identification of previous adverse reactions to different stimuli. A summary of different triggering factors is shown in Table 1. When considering drugs with potential to cause or worsen MC degranulation, selecting a drug that the patient has tolerated

<table>
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<tr>
<th>Table 1. Possible Triggering Factors</th>
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<tr>
<td><strong>Physical stimuli</strong></td>
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<tr>
<td>Heat, cold, friction of skin lesions, pressure, excessive sunlight, exercise</td>
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<tr>
<td><strong>Emotional factors</strong></td>
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<tr>
<td>Stress, anxiety</td>
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<td><strong>Drugs (Examples)</strong></td>
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<tr>
<td>Aspirin and other NSAID*, alcohol, morphine and derivatives, polymyxin-B, amphotericin-B, quinine, some drugs used in general anesthesia (inductors and muscle relaxants- succinylcholine, D-tubocurarine, gallamine, decamethonium), radiographic dyes**, dextromethorphan, β-adrenergic blockers, α-adrenergic and cholinergic receptor antagonists</td>
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<tr>
<td><strong>Venoms</strong></td>
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<tr>
<td>Snakes and insects***</td>
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<tr>
<td><strong>Polymers</strong></td>
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<tr>
<td>Dextran, gelatin</td>
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<tr>
<td><strong>Biologic-response modifiers</strong></td>
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<tr>
<td>Interferon alpha</td>
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<tr>
<td><strong>Miscellaneous</strong>**</td>
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1. For references see text. 2. Responses greatly vary from patient to patient. 3. Patients with known sensitivities must wear a Medic alert bracelet or necklace.

* Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may induce mast cell degranulation in some patients and have proven to be effective as a treatment for others. If patients have not taken these drugs before, treatment must be administered under close medical supervision.

** If x-rays studies are necessary, patients should be pre-medicated with H1 and H2 antihistamines and low molecular weight dyes should be used.

*** Venom immunotherapy may be safely and successfully used in selected patients with mastocytosis and sting anaphylaxis, but may be ineffective or not tolerated in other cases.

**** In individual patients, foods, environmental allergens and other factors may exacerbate the symptoms of mastocytosis. If identified, these triggers should be avoided.

1/ Avoidance of Factors Triggering Mast Cell Mediator Release
in the past is preferable. If a new drug need to be used, premedication with H1 and H2 blockers should be considered, as well as administration of a test dose of the drug in a monitored setting with skill in intubation and emergency treatment available in order to assure an adequate therapy if an adverse reaction develops.

2/ General Anesthesia in Mastocytosis

It has been reported that some drugs used in general anesthesia may induce MC-mediator release [193-195]. The exact incidence of complications in general anesthesia in mastocytosis is not known and there appears to be a great variability in patient response. Despite this, general anesthesia is generally considered as a high risk procedure in mastocytosis since severe reactions such as systemic hypotension/anaphylactoid reactions and coagulopathy even resulting in death have been reported [196-203]. This is particularly important in patients with a history of allergic reactions and known allergies, such as those with a history of adverse reactions to general anesthesia. Protocols aiming at preventing anesthetic complications have been proposed [204-210]. Since β-adrenergic blockers interfere with epinephrine, their use is generally contraindicated for patients undergoing surgery (reviewed in Ref. [81]).

A close communication between anesthesiologists, surgeons, and intensivists must be established prior to surgery. Samples to determine serum tryptase and histamine levels [211,212] may be obtained and blood coagulation parameters should be monitored perioperatively and during anesthesia if suspected mast cell degranulation events occur.

3/ General Management of Pediatric Mastocytosis

Pediatric mastocytosis is histopathologically limited to skin in most patients, although mediators released from skin mast cells can cause symptoms systemically.

In mastocytoma, friction or mechanical irritation of the lesion induces whealing, local pruritus, and sometimes vesicles or bullae. Treatment (Table 2) include a careful control of triggers and, if necessary, topical therapy with sodium cromolyn or corticoids. Use of topical corticosteroids under occlusive dressings should be undertaken with caution because repeated application of corticosteroids may produce cutaneous atrophy. Surgical excision should be considered as an alternative treatment in solitary mastocytomas, according to the severity of the symptoms and resectability of the mastocytoma lesion.
There is a great heterogeneity regarding mast cell-mediator related symptoms in UP; usually, symptoms and signs related to mast cell-mediator release are most prominent at the onset of the disease with a progressive amelioration in following years.

Mild pruritus is the only clinical manifestation in a high percentage of cases [167,213] while in some cases intense pruritus can occur with other symptoms such as flushing, abdominal pain, diarrhea, nausea, vomiting, irritability, hypotension and blistering. Usually, such symptoms are provoked by different triggers (see Table 3) such as physical irritation of the lesions, changes in body temperature, exercise, emotional stress, dentition, or mast cell-degranulating agents such as dextrometorphan [214] or narcotics [215]. It should be noted that, in authors’ experience bacterial or viral infections as well as dentition are frequent triggers for mast cell-mediator release in children. Most children tolerate routine childhood vaccinations well.

Different treatment approaches can be used on the basis of the intensity of symptoms. In a high percentage of cases, avoidance of triggers together with as-needed use of topical cromolyn or corticosteroids may be sufficient. In patients with more pronounced symptoms, the treatment of choice is H1 antihistamines (see Table 2) with or without oral sodium cromolyn and H2 antihistamines in cases with peptic symptoms. Although less common in children, abdominal pain and diarrhea could be treated by using oral sodium cromolyn at a dose of 10 to 20 mg/kg/day, and in cases with partial or no response, NSAIDs (in older children and if they have been previously tolerated) or antileukotrienes may be used with caution.

Patients with DCM must be considered to be at a higher risk for medical emergencies than patients with more limited forms of cutaneous disease. Life-threatening episodes such as vascular collapse or anaphylaxis requiring intensive therapy with epinephrine and other resuscitative measures are more frequent in this category of the pediatric mastocytosis. In patients with diffuse blistering and bullae, an intensive therapy must be started at diagnosis. Such therapy may include a careful local care of the skin with zinc sulphate and topical cromolyn, in intact skin areas, together with measures to prevent infections such as mupirocin in denuded areas. Furthermore, oral therapy with sedating and nonsedating H1 antihistamines, H2 antihistamines, sodium cromolyn, antileukotrienes and if response is not adequate, oral glucocorticoids may be considered. In cases with persistence of blistering with or without vascular collapse, PUVA-therapy should be considered. Self-injectable epinephrine is generally prescribed to be used as needed in case of an unpredictable anaphylactic episode.

**4/ General Management of Adult Mastocytosis**

In addition to avoidance of mediator-releasing triggers (Table 1), treatment of MC-mediator related symptoms in adult mastocytosis depends on the intensity and frequency of symptoms and signs. The mainstays of treatment for most categories of mastocytosis are H1 and H2 blockade with or without mast cell membrane stabilizers and leukotriene inhibitors in order to control the most frequent manifestations such as pruritus, flushing, gastric hypersecretion and diarrhea and, in some cases, to prevent or ameliorate anaphylaxis. Therapeutic protocols may be carefully selected in each individual case and, in authors’ experience, drug dosages should be titrated in order to obtain the most effective regimen with least side effects for each individual patient. A rational approach for the treatment of adults may include oral sodium cromolyn and nonsteroidal H1 antihistamines (loratadine, fexofenadine, desloratadine, cetirizine, levocetirizine, among others) which may be useful in mitigating symptoms. In recalcitrant symptoms such as intense pruritus, data from other groups [81] as well as authors’ experience support the addition of sedating H1 antihistamines (diphenhydramine, hydroxyzine, doxepin), for as-needed or scheduled use. Addition of H2 antihistamines may provide additional symptom control [80,216] (reviewed in Refs. [81,217,218]). H2 antihistamines with or without proton pump inhibitors should be also used to treat gastric hypersecretion or peptic ulcer (Table 3).

Abdominal cramping and diarrhea (Table 3) may be controlled with oral sodium cromolyn in some cases [29,52,148] (reviewed in Refs. [81,217,218]). Recommended adult dosage is 200 mg four times day. Use of leukotriene antagonists (e.g., zafirlukast, montelukast) in recalcitrant cases may be considered (Luis Escribano, personal communication, January 2001).

Treatment of malabsorption (Table 3) should include sodium cromolyn and a careful control of different laboratory parameters to monitor response. In refractory cases positive responses have been described using low to

<table>
<thead>
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<th>Table 3. Treatment of Gastrointestinal Disease</th>
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<tr>
<td><strong>Peptic ulcer disease/gastroesophageal reflux (GERD)</strong></td>
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<td><strong>Abdominal cramping</strong></td>
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<td><strong>Diarrhea</strong></td>
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interferon therapy. Among others, should be undertaken prior to institution of depression, ischemic cardiopathy, and severe hepatopathy, administration of interferon is contraindicated such as the existence of other pathological conditions in which the 

A careful evaluation of side effects as well as considered and positive responses has been described 1.5 to 3 millions of units thrice at week) should be treatment with low to intermediate doses of Interferon (i.e. developing vertebral collapse or other pathological fractures, selected cases with severe osteoporosis who are at risk for development of osteosclerosis [156,229]. In selected cases with severe osteoporosis who are at risk for developing vertebral collapse or other pathological fractures, treatment with low to intermediate doses of Interferon (i.e. 1.5 to 3 millions of units thrice at week) should be considered and positive responses has been described [177,178,228]. A careful evaluation of side effects as well as the existence of other pathological conditions in which the administration of interferon is contraindicated such as depression, ischemic cardiopathy, and severe hepatopathy, among others, should be undertaken prior to institution of interferon therapy.

Diffuse or patchy bone sclerosis is an uncommon manifestation of mastocytosis mainly associated with non-aggressive forms. A few reports suggest that disodium cromolyn may be of added value in specific cases for the improvement of intestinal absorption. In cases with osteopenia therapeutical approaches may include calcium and Vitamin D supplementation and estrogen replacement in postmenopausal women, when indicated. Small case series or case report [39,225-227] as well as personal experience of authors support the utility of biphosphonates in the treatment of osteopenia/osteoporosis associated with mastocytosis. In selected cases with severe osteoporosis who are at risk for developing vertebral collapse or other pathological fractures, treatment with low to intermediate doses of Interferon (i.e. 1.5 to 3 millions of units thrice at week) should be considered and positive responses has been described [177,178,228]. A careful evaluation of side effects as well as the existence of other pathological conditions in which the administration of interferon is contraindicated such as depression, ischemic cardiopathy, and severe hepatopathy, among others, should be undertaken prior to institution of interferon therapy.

| Table 4. Treatment of Bone Disease Secondary to Mast Cell-Mediator Release |
|-----------------------------------------------|-----------------------------------------------|
| Osteopenia/osteoporosis                       | Cromolyn sodium                               |
|                                               | Calcium supplementation ± vitamin D           |
|                                               | Biphosphonates                                |
|                                               | Consider estrogen therapy for postmenopausal women, testosterone replacement in men with low testosterone levels |
|                                               | Consider interferon α-2b in patients with severe osteoporosis at risk for pathological bone fractures |
| Diffuse bone sclerosis                        | Cromolyn sodium                               |
|                                               | Low doses of corticoids                       |

intermediate doses of oral prednisone [219-224] (reviewed in Refs. [81,218]). Such patients should be maintained on the lowest possible dose of glucocorticoid which is able to control symptoms.

The most important goal in the treatment of osteopenia (Table 4) is the early diagnosis before osteoporosis develops. Malabsorption could, at least theoretically, impair calcium absorption and sodium cromolyn therapy could be useful for the improvement of intestinal absorption. In cases with osteopenia therapeutical approaches may include calcium and Vitamin D supplementation and estrogen replacement in postmenopausal women, when indicated. Small case series or case report [39,225-227] as well as personal experience of authors support the utility of biphosphonates in the treatment of osteopenia/osteoporosis associated with mastocytosis. In selected cases with severe osteoporosis who are at risk for developing vertebral collapse or other pathological fractures, treatment with low to intermediate doses of Interferon (i.e. 1.5 to 3 millions of units thrice at week) should be considered and positive responses has been described [177,178,228]. A careful evaluation of side effects as well as the existence of other pathological conditions in which the administration of interferon is contraindicated such as depression, ischemic cardiopathy, and severe hepatopathy, among others, should be undertaken prior to institution of interferon therapy.

Diffuse or patchy bone sclerosis is an uncommon manifestation of mastocytosis mainly associated with non-aggressive forms. A few reports suggest that disodium cromolyn may be of added value in specific cases for the treatment of pain associated with osteosclerosis [156,229]. Furthermore, marked decrease in serum tryptase in patients with diffuse sclerosis undergoing sodium cromolyn therapy has been observed (L. Escribano, personal communication) as well a decrease in bone density as assessed by both X-rays of bones and bone densitometry (R. Núñez, personal communication). Responses to corticosteroids have been observed using doses of 0.3 mg/Kg and day with tapering to 0.2 to 0.3 mg every other day (Rosa Núñez, personal communication, May 2004).

Mastocytosis associated with recurrent vascular collapse or anaphylaxis represents a therapeutic challenge (Table 5). Acute or impending episodes of vascular collapse should be treated with epinephrine (Epi-Pen). Preventive treatments have variable success and generally include the combined use of sedating and non-sedating H1 antihistamines together with H2 antihistamines, NSAIDs, anti-leukotrienes and glucocorticoids. Ketotifen has been described to be beneficial in a subset of patients with idiopathic anaphylaxis, however its value in mastocytosis is not clear. Doxepin, a psychotherapeutic agent with antihistamine properties, may be effective especially in cases with neuropsychiatric symptoms. In selected cases unresponsive to conventional therapy suffering from life-threatening episodes, low doses of alpha interferon may be used. Initial doses must be administered under medical supervision with a target dose of 1 to 1.5 millions units two to three times at week.

**Hymenoptera Venom Sensitivity and Mastocytosis**

Patients with mastocytosis and anaphylaxis after Hymenoptera stings have been treated with venom immunotherapy with varied success and shown protection after re-stinging [230]. However, two patients with mastocytosis who received venom immunotherapy died from a sting after massive mast cell mediator release [231], indicating a variable degree of protection with venom immunotherapy depending on the mast cell burden and/or the extent of mast cell releasability and mediator release. Immunotherapy should not be administered without evidence of specific IgE-mediated sensitization to hymenoptera venom. Evidence of anaphylaxis after hymenoptera sting in patients with negative skin test to hymenoptera venom may

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<tr>
<th>Table 5. Treatment of Hypotension/Anaphylactoid Reactions</th>
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<tr>
<td>Strict avoidance of triggers</td>
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<tr>
<td>Acute life-threatening events:</td>
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<td>Treatment at the ICU</td>
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<tr>
<td>Maintenance in recurrent episodes</td>
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<tr>
<td>(see also diffuse cutaneous mastocytosis)</td>
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suggest that mast cell activation can be induced through non IgE mechanisms in patients with mastocytosis. In a recent paper including 259 Hymenoptera venom-allergy patients, elevated basal serum tryptase levels were found in 19 cases and 3 of 16 patients in this group was documented to have cutaneous mastocytosis by skin biopsy [232].

FUTURE PERSPECTIVES

Mastocytosis remains to be an incurable disease at the present time for most patients. Moreover, it is often difficult to achieve a complete symptomatic control in many patients. This is probably because of the lack of knowledge about a complete array of mediators released from the mast cells as well as the inefficacy of the currently available drugs to target all known mast cell mediators. It is likely that not all patients with mastocytosis will be candidates for mast cell cytoreduction even if a drug capable of reducing mast cell numbers is developed. It is thus desirable to develop new therapeutic modalities not only targeting the mast cell itself but also its mediators to achieve better symptomatic control in those who are not candidates for mast cell cytoreduction.

Histamine Receptor Modulation

Histamine is one of the best studied mast cell mediators, which results in a wide array of tissue responses through binding to various receptors. As of now, four histamine receptors have been identified. H1 receptors generally mediate the inflammatory effects of histamine, while H2 receptors are best recognized for their involvement in gastric acid secretion; antagonists for both H1 and H2 receptors have long been available for clinical practice, and their use in mastocytosis is discussed above. On the other hand, there are no FDA-approved drugs to modulate H3 or H4 receptors.

H3 receptors were identified in 1983 [233], and the human receptor was cloned 1999 [234]. They are predominantly found in the central nervous system (most notably in thalamus and the caudate nucleus) and peripheral nervous system in presynaptic neurons, where they are involved in modulation of sympathetic and parasympathetic responses [235]. They also play important roles in regulation of gastric acid secretion as they are found on cholinergic neurons of the myenteric plexus and in the endocrine cells of the gastric mucosa [236]. Stimulation of H3 receptors results in inhibition of secretion of a number of neurotransmitters including histamine and serotonin [233,237]. Therefore, in contrast to H1 and H2 receptors, efforts of drug development have focused on both agonists and antagonists of the H3 receptors [238]. H3 receptor-modulating drugs may potentially find a wide range of applications in such diverse areas as management of neuropsychiatric, gastrointestinal and vascular symptoms.

H4 receptors have more recently been identified by several groups [239-244]. H4 histamine receptors are mainly expressed on hematopoietic cells including mast cells, although their precise functions are yet to be fully understood. Specific antagonists of the H4 receptor have recently been developed [245,246]; one such inhibitor is shown to block histamine-induced mast cell and eosinophil chemotaxis [246,247].

Tryptase and Chymase Inhibitors

The potential therapeutic value of protease inhibitors depends upon the clinical role that mast cell proteases play in systemic mastocytosis. Total tryptase levels are markedly elevated in the circulation and, presumably, in the tissues of those with mastocytosis. However, enzymatically inactive precursors account for most of this tryptase, and these molecules are unlikely to be biologically active. Nevertheless, release of active forms of tryptase, chymase, cathepsin G and carboxypeptidase A3 could be involved in the tissue remodeling associated with bone, liver and other organ system manifestations. The ultimate therapeutic intervention will be to kill the deviant mast cells and their precursors. Without such an intervention, whether inhibitors of these proteases will provide benefit beyond current mediator pharmacological agents is unproven.

Antibodies

CD25 (the alpha chain for the IL-2 receptor) is expressed on activated lymphocytes and neoplastic mast cells of patients with mastocytosis, but not on normal mast cells [248], and the plasma level of CD25 is elevated in patients with mastocytosis [249]. Therefore, in addition to its role in diagnosis of mastocytosis, CD25 constitutes a reasonable therapeutic target. Strategies targeting CD25 on lymphocytes or neoplastic cells have been successfully exploited as immunosuppressant or cytotoxic therapies respectively [250].

Chimeric (humanized) antibodies consisting of murine fragments recognizing the IL-2 receptor alpha chain fused to human IgG1 constant chain aim to block binding of IL-2 to its receptor with minimizing the immune response against a mouse protein [251]. However, such molecules have unknown therapeutic potential in mastocytosis as it is not clear whether the CD25 molecule is functionally important in neoplastic mast cell survival.

Another approach is the use of recombinant immunotoxins containing a CD25-binding protein (such as the variable portion of an anti-CD25 antibody or IL-2 molecule) linked to a toxin [252]. IL-2 sequences 1-133 linked to diphtheria toxin fragments A and B (denileukin difitox) has been approved for treatment of cutaneous T-cell lymphoma expressing CD25 [253], while other recombinant immunotoxins are undergoing evaluation in clinical trials. One such immunotoxin consisting of Pseudomonas aeruginosa exotoxin A linked to the Fv fragment of an anti-CD25 antibody produced major or partial responses in patients with hairy cell leukemia and other CD25+ hematologic malignancies who were refractory to standard therapies [254,255], and reduced mast cell numbers in bone marrow cultures obtained from patients with mastocytosis (Akin et al. unpublished observation). However, several potential problems may prevent consideration of immunotoxins for many patients with mastocytosis. Use of immunotoxins may be complicated by life-threatening adverse reactions such as hepatotoxicity and vascular leak syndrome. It should also be noted that expression of CD25 in neoplastic MC may be downregulated during evolution in patients with advanced categories of mast cell disease such as mast cell leukemia [256].
Finally, neutralizing antibodies against the bacterial toxin or the recombinant molecule may develop with repeated infusions and reduce the efficacy of the drug.

**Mutated Kit as a Therapeutic Target**

Activating codon 816 mutations carried by most patients with mastocytosis render the mast cells resistant to the currently available tyrosine kinase inhibitor imatinib. However, rare patients with mast cell disease may have a wild type c-kit or c-kit carrying activating mutations outside of tyrosine kinase domain are predicted to respond to imatinib. Morphological analysis of mast cell infiltrates may be helpful, as a recently described patient with a disease variant characterized by well-differentiated, round and fully granular mast cells lacked the codon 816 mutation and carry a fusion gene involving PDGFRα which is also highly sensitive to imatinib [257,258].

Patients with aggressive disease variants who carry codon 816 c-kit mutations are candidates for investigational therapies evaluating novel Kit inhibitors. A novel tyrosine kinase inhibitor, PKC412, has been shown to preferentially inhibit Kit with codon 816 mutations, and produced a transient response when administered to a patient with mast cell leukemia [259]. 17-AAG, another compound leading to degradation of mutated Kit by inhibiting its association with the heat shock protein Hsp90 showed in vitro cytotoxic activity against patient bone marrow mast cells [260]. Clinical efficacy of these and other similar compounds remains to be evaluated by clinical trials in patients with advanced forms of mastocytosis.

**ACKNOWLEDGEMENTS**

All authors contributed equally to the design, content, and final approval of the paper.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbr</th>
<th>Meaning</th>
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<tr>
<td>AA</td>
<td>Arachidonic acid</td>
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<tr>
<td>PLA</td>
<td>Phospholipase</td>
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<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
<td>PG</td>
<td>Prostaglandin</td>
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<tr>
<td>NSAIDs</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
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<tr>
<td>LT</td>
<td>Leukotriene</td>
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<tr>
<td>LO</td>
<td>Lipooxygenase</td>
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<tr>
<td>PAF</td>
<td>Platelet activating factor</td>
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**REFERENCES**
