# Insights on the inhibitors of dipeptidyl peptidase 1 as mast-cell stabilizer

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**Background**
Tryptase and chymase are potent proteases secreted by mast cells upon activation. These abundant mast-cell proteases are converted into catalytically active forms by dipeptidyl peptidase 1 (DPP1). DPP1 is one of the most important components to initiate chain reaction for triggering the inflammatory response mediating allergic diseases such as asthma and allergic rhinitis. DPP1 can remove the safety catch on various mast-cell proteases, including tryptase and chymase, and plays an essential role in controlling their activity. However, the exact role of DPP1 in mast-cell degranulation is still not fully recognized.
DPP1 may help mast cells degranulate via acting inside the cell or after secretion. DPP1 inhibitors are essential for mast-cell stabilization. In this review, we will discuss the contribution of DPP1 in mast-cell degranulation and the role of DPP1 inhibitors in mast-cell stabilization, which may help finding new therapeutic strategies for asthma.

**Keywords:**
dipeptidyl peptidase 1, inhibitors of mast cell, mast-cell-stabilizing compounds

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## Introduction

In asthma, mast-cell activation is a common occurrence [1,2]. This is linked to the secretion of a group of proteases that are stored in mast-cell intracellular granules. Mast-cell proteases are emerging as significant mediators of inflammation and tissue remodeling and can have powerful effects once they are released. Tryptase and chymase are among the most potent mast-cell proteases [3].

Drug development has shifted its focus to inhibiting tryptase and chymase after release into the lungs. Dipeptidyl peptidase 1 (DPP1, also known as cathepsin C, CTSC) is a cysteine protease that has been associated with the processing of mast-cell proteases. It is critical to study its ability to regulate the expression and release of active mast-cell proteases, as well as its role in the inflammatory process that will help finding new treatments for asthma. Tryptase and chymase are produced as inactive proenzymes with a dipeptide at the N-terminus that distinguishes them from the mature enzyme. DPP1 has been shown to activate recombinant proforms of tryptase [4] and chymase [5]. In addition, mast cells in DPP1-mutant mice have been found to exhibit a completely defective chymase activity (but immunoreactive chymase expression was normal), while tryptase activity was significantly reduced [6,7]. Despite being known as an intracellular enzyme, immunohistochemistry studies on dog tissues have revealed that DPP1 is stored within mast-cell granules and released upon degranulation [8]. This finding was supported by previous studies that reported the ability of canine DPP1 to cleave specific extracellular-matrix proteins [9] and that bovine DPP1 can create peptide agonists for human melanocortin receptors from human α-melanocyte-stimulating hormone [10]. Exploring the functions of DPP1 will provide useful information on its role in mast-cell protease processing and other mediator activities in asthma. Therefore, the goal of this review is to discuss the contribution of DPP1 in mast-cell degranulation. We will also review the inhibition of mast-cell production of active forms of proteases such as tryptase and chymase via DPP1 inhibitors, which may help finding new treatment strategies for asthma.

### Mast cells

Mast cells are multifunctional tissue-dwelling cells that play a major role in allergies and other innate-immune systems. Mast cells are divided into two types: $M_{CT}$ cells, which produce tryptase only, and $MC_{TC}$ cells, which produce both chymase and tryptase. $M_{CT}$ cells are related to the immune system, whereas $MC_{TC}$ cells are nonimmune-system related [11].

Within mast cells, there are secretory granules that swell and release their contents upon activation of the cells. Mast cells become activated when two or more adjacent molecules cross-link high-affinity IgE receptors on their surface. As a result of this activation, protease molecules such as tryptase, chymase, and histamine are secreted from the granules within the mast cells. This process is known as mast-cell degranulation that results in an inflammatory response that takes place in allergies and related disorders such as asthma [12].

#### Asthma

Asthma is a chronic lung disease in which epithelial cells collaborate with the innate and adaptive immune systems to generate airway hyperresponsiveness, mucus overproduction, airway-wall remodeling, and bronchoconstriction. Recurrent episodes of wheezing, dyspnea, and chest tightness characterize it clinically [13]. Cells that line the bronchi become chronically inflamed due to their hyperresponsiveness to certain stimuli [14]. When becoming in contact with the stimulus, contraction occurs, resulting in breathing difficulty and increased mucus production. Breathing difficulty is associated with wheezing sound and a feeling of constriction of the chest and coughing. This response only occurs as long as the stimulus is present and recurs at intervals [14].

There are several environmental factors that cause the incidence of asthma, including respiratory-tract infections, allergens (particles that cause an allergic response such as house dust, mite droppings, and pollen), smoking, exercise, cold air, emotional upsets, and chemical irritants [15].

There are two phases of an asthma attack: the early phase and the late phase. The early phase is marked by bronchoconstriction followed by a decrease in the effect of forced expiratory volume, while the late phase is characterized by eosinophil inflammation. Activated mast cells are major contributors to the pathophysiology of asthma. Mast cells infiltrate to the smooth muscles of the airway, the mucous glands, and the bronchial epithelium. Mast cells secrete a wide range of factors that enhance asthma pathogenic events such as bronchoconstriction, mucosal edema, and mucus secretion. These factors include histamine, prostaglandin D2, leukotriene C4, and others [16]. In bronchial asthma, histamine is released from mast cells into the airway surface as a result of allergic reactions. Antihistamines have been found to have bronchodilating properties, inhibitory effects on allergen activity preventing the allergen-induced nonspecific airway hyperresponsiveness [17,18]. In addition, mast cells release multiple proinflammatory cytokines, such as interleukin (IL)-4, IL-5, and IL-13, which mediate IgE production and eosinophilic inflammation. Importantly, activated mast cells secrete a range of proteases, the most important of which are tryptase and chymase. These proteases contribute to several determinal actions in asthma [19].

#### Mast-cell proteases

Proteases are proteins produced by mast cells that are kept in membrane-enclosed intracellular granules, until degranulation is triggered. IgE cross-links the high-affinity IgE receptor with a multivalent allergen, causing mast-cell degranulation. Understanding the functional impact of proteases is crucial and distinguishing between their roles in laboratory animals and humans is required to determine the degree of confidence in data obtained from animal-model studies [20].

The inflammatory potential of mast-cell proteases was the first biological aspect to be studied, and it has received great attention. However, proteases can play dual roles. They contribute to allergic inflammation, and on the other hand, they can also act as anti-inflammatory mediators. This may explain the contribution of mast cells to allergies as well as tissue homeostasis [21]. For example, tryptases are considered markers of local and systemic mast-cell degranulation and anaphylaxis [22], on the other hand, they play a protective role against serious bacterial lung infections and reduce the 'rubor' component of inflammation caused by vasodilating neuropeptides [21]. Mast-cell chymases also can act as anti-inflammatory mediators that help maintain intestinal barrier integrity and expel parasitic worms during anaphylaxis by generating angiotensin II, and help to maintain blood pressure and expel parasitic worms. Carboxypeptidase A3 along with other mast-cell proteases can reduce systemic toxicity of endogenous peptides like endothelin and neurotensin and inactivate venom-associated peptides during septic peritonitis.

In addition, mast-cell proteases affect nonmast-cell proteases through activating matrix metalloproteinase cascades, which are important in infection responses and tissue-injury repair.

Both tryptase and chymase are secreted in their active forms after being stimulated by CTSC (DPP1) during vesicle maturation [23].

### Tryptase

Tryptase is the most important protease in mast cells. It is a serine protease stored in secretory granules and secreted together with numerous other mast-cell products upon activation. It is a main actor in mast-cell-mediated functions and could be used as a mast-cell activation indicator. There are no inhibitors for tryptase since its active tetramer dissociates quickly in the absence of heparin, which is required for the stability of tryptase in its active tetrameric form [24].

Tryptase was found to be specific to mast cells, and thus, it was suggested that they can be used as a tool to identify human mast cells. In addition, tryptase detection by its specific antibodies may be useful for distinguishing between these cells and basophils [25].

Human tryptase is divided into three types: α-tryptase, β-tryptase, and γ-tryptase. β-tryptase is the active form and acts outside the mast cell, whereas α-tryptase and γ-tryptases are generally inactive. So, β-tryptase is considered the main form of the enzyme involved in the inflammatory events that occur when mast cells are activated [22]. That is why the tryptase has been recognized as a marker for allergy, which is released locally, as in asthma, or systemically, as in anaphylaxis.

The serum tryptase levels of allergic patients were tested after induction of systemic anaphylaxis reaction to a bee sting that began to rise ~30 min after induction and peaked 1–2 h later. After this time, levels begin to decline, having a half-life of around 2 h [26].

The levels of β-tryptase in the blood of femoral vessels were found to be higher in the postmortem of fatal anaphylaxis than in most other death causes [27]. Similarly, in cases with sudden infant-death syndrome, tryptase levels were found to be higher than in those who died due to other causes and this was considered to be a marker of anaphylaxis [28]. The use of tryptase measurements in the diagnosis of anaphylaxis was not particularly sensitive, whereas high tryptase levels indicate anaphylaxis, but low levels do not rule out the possibility of a diagnosis. On the other hand, higher tryptase levels can be observed in a variety of illnesses; thus, additional considerations such as the patient history should be addressed. The use of tryptase assays in identifying anaphylaxis, especially as a result of food, is widely accepted [29].

Furthermore, elevated tryptase levels were found in a number of nonanaphylactic cases who had high baseline levels. Tryptase measurements were therefore found to be more sensitive and specific when serial measurements were taken [30].

Nearly one-fifth of patients with acute-allergy symptoms tested in an emergency room had higher serum β-tryptase levels, with urticaria and tachycardia being the most prevalent symptoms. Although the fact that high β-tryptase levels were uncommon, they were found ~10 times more frequently in people who had hypotension, tachycardia, or wheezing than in people who did not have such symptoms. It is possible that high β-tryptase levels are more symptomatic of severe allergic disease since only a few people are diagnosed with anaphylactic shock [31].

Interestingly, patients with sensitivity to food allergens do not exhibit high serum or saliva tryptase levels. In a study by Vila *et al.* [32], serial serum and saliva tryptase measurements were recorded before and after food challenges in patients with previous systemic reactions to food. Only 25% of patients with a positive food challenge had elevated serum tryptase levels. There was no increase in tryptase levels in patients who did not react to the food challenge and saliva tryptase was undetectable in nearly all other patients, including controls. However, food antigens given intraluminally induced higher levels of intestinal tryptase, as well as histamine, peroxidase, and prostaglandin D2 in food-allergy sufferers than in controls. Plasma tryptase exhibited similar levels in both food-allergic and controls, and did not rise by antigen challenge [33].

### Chymase

Chymase is a serine protease, similar to tryptase. It is secreted by mast cells and retained in lesser amounts within the secretory granules [34]. Chymase is thought to be found mostly in the connective-tissue subtype of mast cells ($MC_{TC}$), however, it is also likely to be present in mucosal tissue mast cells ($MC_T$) [35].

The pH of the mast-cell granule that stores chymase is reported to be 5.5. At this pH, chymase was found to be inactive, it is converted into its active form after being released. Thus, chymase is synthesized in an inactive preprotein form, then converted within the mast cell into its mature form, and when released, it becomes quickly active due to the absence of the pH-suppressive effect outside the mast cell. During the maturation process, the inactive form, prochymase, is catalyzed by the enzyme DPP1 via removal of a dipeptide to be converted into its mature chymase form [8].

Through the cleavage of collagen IV and the breaking of the dermal–epidermal interface, chymase aids in the remodeling of the airways in asthma and chronic obstructive pulmonary disease, which is suspected to cause mucus hypersecretion [11]. It is also linked to mucosal inflammation, as evidenced by the activation of IL-1 signaling, the degradation of IL-4, and the activation of submucosal gland secretions [36]. Following the exposure to an allergen, chymase, along with other mast-cell products such as heparin, histamine, and tryptase, is produced in mast cell and released by degranulation [37]. Chymase is assumed to be responsible for a number of inflammatory processes, including microvascular leakage, neutrophil buildup, activation or inactivation of numerous inflammatory cytokines, and stimulation of mucus secretion [19].

Several types of human chymase were discovered using affinity chromatography [38], which showed differences in tissue distribution, it was proposed that the possibility of chymase action changes at distinct inflammatory sites. Upon allergin exposure, chymase is released from mast cells and acts to promote inflammatory processes, implying that it plays a major role in allergic reactions and anaphylaxis. Additionally, chymase was found in cardiac blood-derived serum in all deaths caused by anaphylaxis in a postmortem research, but in only less than 2% of deaths of other reasons. Furthermore, all anaphylactic deaths had a positive correlation between serum chymase and tryptase levels. Drugs, such as antibiotics and anesthetics, were found to be the cause of the majority of anaphylactic reactions [21].

### Dipeptidyl peptidase 1

DPP1 is a cysteine protease found in the cytoplasmic secretory granules of bone marrow-derived leukocytes like myelomonocytic cells, cytotoxic T cells, and mast cells. DPP1 has been suggested to have an extracellular function. The roles of DPP1, as demonstrated by in-vitro studies, include degradation and turnover of proteins, and activation of enzymes, including tryptase and chymase. DPP1 may also have a role in the growth and differentiation of mast cells [39]. DPP1 is also known as CTSC. It is a lysosomal cysteine protease enzyme involved in the conversion of proenzymes to their active forms. Granzymes A and B, mast-cell chymase, granzyme K, and thrombin-cleaved plasminogen activator are all processed by this enzyme [40]. CTSC is found in all tissues, although its level is higher in the lungs, kidneys, placenta, liver, spleen, and intestines [41]. DPP1 has the potential to produce allergic reactions where it has been discovered as a probable mast-cell activator that contributes to various allergic reactions [8]. DPP1 is involved in the inflammatory process of asthma. It is involved in matrix-protein turnover and remodeling of the airways in asthma. DPP1 was found to cleave various extracellular-matrix proteins [42].

### Structure

DPP1 or CTSC is a tetramer, which means having four identical subunits. Heavy, light, and prodomains are the three chains that make up each subunit. The prodomain has been investigated for potential sulfide bond and glycosylation sites. CTSC is generated in prostructure and so requires cleavage by cathepsin L (CTSL) or cathepsin S (CTSS) to become active [43]. The 35-kD protein was originally considered to be coded for by two exons on chromosome 11 with one intervening intron. However, it was discovered that this area actually had seven exons, two of which were placed in the previously identified exon 2, despite the fact that both transcripts encoded the same polypeptide. A smaller alternatively spliced version with only two exons and 31 amino acids has also been identified [44].

### Expression and function

The DPP1/CTSC protein expression is boosted by two different T-helper type-2 cytokines (Th-2): IL-4 and IL-13. Both of these Th-2 ILs are potent stimulators of the expression of the two DPP1/CTSC variants although the expression of the smaller variant is increased by these Th-2 molecules in epithelial cells ten times higher than that of wild-type cells [45]. IL-4 and IL-13 are also detected in human bronchial epithelial cells supporting the theory that these cells could be a source of DPP1/CTSC [8]. IL-2 stimulates DPP1/CTSC expression in lymphocytes. DPP1/CTSC is found with high levels in the kidney, placenta, and lymph nodes [8]. The DPP1/CTSC class of proteins plays a crucial role in the degradation of proteins and as a coordinator of serine protease activity within immunological and inflammatory cells. DPP1/CTSC is also detectable in the brain [46]. DPP1/CTSC is thought to be modified posttranslationally via disulfide bonds or glycosylation [45]. DPP1 can activate different serine proteases by removing dipeptides from the N-termini of the proprotease protein substrates [47].

### Dipeptidyl peptidase 1 inhibitors

DPP1 action can be inhibited by various inhibitors such as diazomethylketone (Gly–Phe–DMK),

Gly–phe–CHN2, acyloxymethyl ketone, and vinyl sulfones (Ala–Hph–VS-Ph), causing inhibition of the serine protease activities [47].

Pretreatment of the U937 and EcoM-G cells with the DPP1 inhibitor Gly–Phe–DMK resulted in a restriction of cut fragments and the level of enzymatic activity was reduced. The molecules that were measured for enzymatic activities were neutrophil elastase, cathepsin G, and proteinase-3 [48]. Moreover, the DPP1 inhibitor Gly-phedisazomethane (Gly–phe–CHN2) was found to block trypsin maturation in the human mast-cell line HMC-1 [49]. Acyloxymethyl ketones are moderate inhibitors of cellular DPP1, which can inhibit DPP1 but in a slow manner. Vinyl sulfones (Ala–Hph–VS–Ph) are among the best-known DPP1 inhibitors. They are able to inhibit intracellular DPP1, and are considered as the most stable inhibitors that act with high potency [47]. Importantly, the most potent and highly selective inhibitors described till now still exhibit poor stability, which is most likely due to their peptidic structure [50].

## Conclusion

The cysteine protease DPP1, also known as CTSC, can be found in a number of organs, including lungs. The most prevalent source of DPP1 in lungs is mast cells. Mast cells have DPP1 in their cytoplasmic granules. DPP1 is capable of inducing endoproteolytic cleavage of extracellular-matrix proteins. The identification of DPP1 in airway mast cells and its ability to cleave matrix proteins indicated that DPP1 may be involved in mast-cell-mediated matrix-protein turnover and airway remodeling in chronic airway diseases such as asthma. Although some proteases such as mast-cell tryptase and chymases cause allergic inflammation and may be targeted to treat asthma or other allergic diseases, however, they play a protective role and can exert an anti-inflammatory action in some conditions. Limiting mast-cell synthesis of active forms of these proteases has been proposed as a trail for treatment of asthma. DPP1 contributes to processing of mast-cell proteases. It has an ability to regulate the development and release of active mast-cell proteases, as well as its contribution to the pathogenic process in asthma. DPP1 inhibitors are vital for mast-cell stabilization and may serve as therapeutic tools for treatment of asthma.

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**Conflicts of interest**

There are no conflicts of interest.

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