

Involvement of chymase in allergic conjunctivitis of guinea pigs



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ABSTRACT

It has been reported that chymase activity was increased in allergic conjunctivitis patients and this activity was correlated with the severity of the disease. However, the precise roles of chymase in allergic conjunctivitis are unclear, and whether chymase inhibitors are effective for allergic conjunctivitis has not been reported even in experimental animal models. In this study, the roles of chymase in the pathogenesis were evaluated using a selective chymase inhibitor, ONO-WH-236, in a guinea pig model of allergic conjunctivitis induced by cedar pollen. Sensitized guinea pigs were challenged by the pollen, followed by assessing redness and edema in the conjunctiva, and counting the frequency of eye scratching as an itch-associated response. Treatment with the ONO-WH-236 (40 and 80 mg/kg, p.o.) dose-dependently inhibited the induction of redness, edema and scratching behavior. An anti-histaminic drug, ketotifen (3 mg/kg, p.o.), also significantly inhibited conjunctivitis symptoms. Chymase activity was increased in ophthalmic lavage fluid immediately after the pollen challenge. The increase in chymase activity was inhibited by in vivo treatment with ONO-WH-236. Interestingly, increased histamine in the ophthalmic lavage fluid immediately after the challenge was also inhibited by the chymase inhibitor. Administration of human recombinant chymase by eye dropping (0.09 and 0.9 µg/eye) dose-dependently induced scratching behavior, which was inhibited by not only ONO-WH-236 but also ketotifen; however, chymase administration induced only weak redness in the conjunctiva, which was resistant to treatment with anti-histaminic drugs. In conclusion, it was suggested that chymase was released from mast cells after antigen challenge, followed by the induction of conjunctivitis symptoms through histamine release from mast cells. Thus, chymase could be a potential target for pharmacotherapy for allergic conjunctivitis.

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1. Introduction

Mast cells in human tissues are classified into 2 types, MC_{TC} expressing tryptase, chymase and carboxypeptidase A, and MC_T expressing only tryptase (Caughey, 2007; Stevens and Adachi, 2007; Pejler et al., 2010). Among the mast cell proteases, chymase has the characteristic feature of being a chymotrypsin-like serine protease, which is present in the secretory granules of mast cells. It is generally known that upon anaphylactic stimulation, mast cells release chymase together with co-stored proteases, tryptase and

carboxypeptidase A, as well as a pre-formed mediator, histamine. Regarding pro-inflammatory functions, chymase can be a chemical mediator that induces the recruitment of inflammatory cells because intradermal injection of human chymase caused the accumulation of neutrophils and eosinophils in guinea pigs (He and Walls, 1998). This chemoattractant activity of chymase was also confirmed in vitro experiments (Tani et al., 2000; Terakawa et al., 2006). In addition, it has been reported that chymase activated pro-interleukin (IL)-1β to IL-1β (Mizutani et al., 1991), and pro-IL-18 to IL-18 (Omoto et al., 2006), suggesting that chymase could indirectly promote inflammatory responses. Furthermore, interactions between chymase and histamine in anaphylactic responses in mast cells have been demonstrated. It has been reported that anaphylactic histamine release from human and rat mast cells was effectively suppressed by suppression of chymase activity (Katunuma et al., 1986; He et al., 1999, 2004). To date, chymase inhibitors have been developed, and assessed in various models of diseases. In

Abbreviations: AMC, 7-amino-4-methylcoumarin; CIS, conjunctivitis intensity score; DMSO, dimethyl sulfoxide; IL, interleukin; MC_T, tryptase-positive mast cells; MC_{TC}, tryptase-positive, chymase-positive mast cells; NO, nitric oxide; NOS, nitric oxide synthase; OLF, ophthalmic lavage fluid; PAR, protease-activated receptor.

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chronic atopic dermatitis models, chymase inhibitors effectively prevented eosinophil accumulation, formation of edema in the skin, and the itch-associated scratching response (Watanabe et al., 2002; Terakawa et al., 2008).

On the other hand, in a typical immediate-type allergic disease, allergic conjunctivitis, chymase activity in the tears of vernal keratoconjunctivitis patients was significantly higher than in healthy subjects (Ebihara et al., 2004). The level of chymase activity correlated well with the severity of the disease (Ebihara et al., 2004). The predominant type of mast cells in the substantia propria of human conjunctival tissue was MC_{TC} (Irani et al., 1990). In addition, rat mast cell chymase elicited itch in human skin (Hägermark et al., 1972). Andoh et al. (2012) indicated that allergic itch induced by ragweed pollen in sensitized mice was completely dependent on mast cells, whereas involvement of histamine in it was partial. Thus, it has been strongly suggested that chymase is involved in the pathogenesis of allergic conjunctivitis, and that inhibition of chymase can be a therapeutic strategy for the disease. However, there has been no report demonstrating the efficacy of chymase inhibitors on allergic conjunctivitis even in experimental animal models, and the pathophysiological roles of chymase in allergic conjunctivitis have been unclear.

In order to analyze the mechanisms underlying allergic conjunctivitis, we have developed and utilized a guinea pig model of Japanese cedar pollen-induced allergic conjunctivitis (Yasuda et al., 1999; Fukushima et al., 2003; Katayama et al., 2005; Nagata et al., 2008). In the experimental model, when sensitized guinea pigs were challenged by dropping pollen into the eyes once a week, histamine-dependent conjunctivitis symptoms, redness and edema in the conjunctiva, and itch-associated behavior were evoked in the 1st–3rd challenges (Fukushima et al., 2003). In this study, the effect of a chymase inhibitor, ONO-WH-236, on pollen-induced conjunctivitis was examined in comparison with an anti-histaminic drug, ketotifen. In addition, interaction between chymase and histamine was assessed using exogenously applied chymase-induced conjunctivitis, and histamine levels were measured after allergen and chymase challenges. Consequently, we could obtain data indicating that chymase is involved in the induction of allergic conjunctivitis symptoms directly or indirectly through histamine release.

2. Materials and methods

2.1. Effect of chymase inhibitor on chymase activity *in vitro*

ONO-WH-236 (Ono Pharmaceutical Co. Ltd., Osaka, Japan) was used as the chymase inhibitor and its effect on human chymase activity was assessed. N-Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (final concentration 2.5 mM; Sigma–Aldrich, St. Louis, MO, USA) was incubated with various concentrations of ONO-WH-236 or the vehicle, 5% (final concentration) dimethyl sulfoxide (DMSO) in an assay buffer, 50 mM Tris–HCl, 150 mM NaCl, 50 U/ml heparin (pH 7.6) for 10 min. After pre-incubation, human chymase (EastCoast Bio, Inc., North Berwick, ME, USA) was added at a final concentration of 0.2 µg/ml, and then liberated *p*-nitroaniline was measured by photometric determination at 405 nm for 5 min.

To assess the specificity of ONO-WH-236 on chymase activity, its effect on chymotrypsin activity was also evaluated. N-Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (final concentration of 250 µM) was incubated with various concentrations of ONO-WH-236 or the vehicle, 5% DMSO in an assay buffer, 100 mM Tris–HCl, 10 mM CaCl₂ (pH 7.8) for 10 min. After pre-incubation, α-chymotrypsin from bovine pancreas (Sigma–Aldrich), which was dissolved in 2 mM HCl, was added to the mixture at a final concentration of 0.005 units/ml,

followed by kinetic measurement of liberated *p*-nitroaniline by photometric determination at 405 nm for 5 min.

2.2. Animals

Male 3-week-old Hartley guinea pigs were purchased from Japan SLC (Hamamatsu, Japan). Animals were housed in an air-conditioned room at 22–24 °C and 50–70% humidity, with lights on from 08:00 to 20:00. Access to standard laboratory diet and water was provided *ad libitum*. Study protocols of allergic conjunctivitis in guinea pigs were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.3. Allergic conjunctivitis

As previously described (Yasuda et al., 1999; Fukushima et al., 2003; Katayama et al., 2005; Nagata et al., 2008), guinea pigs were sensitized by intraperitoneal injections with an extract of Japanese cedar pollen plus Al(OH)₃ (10 µg pollen protein and 20 mg Al(OH)₃/ml per animal) twice within a week. Two weeks after the second sensitization, animals were challenged by dropping a pollen suspension (2 mg pollen/10 µl/eye) without Al(OH)₃ into each of their eyes. The challenge was conducted once a week until the 3rd challenge.

2.4. Chymase-induced conjunctivitis

Chymase human recombinant (Sigma–Aldrich) was administered at doses of 0.09 and 0.9 µg/10 µl/eye by dropping into both eyes of sensitized guinea pigs on the 7th day after the 2nd or 3rd challenges. These doses of human chymase were comparable to those in another guinea pig study (He and Walls, 1998), in which 0.25 and 2.5 µg/site of human chymase was intradermally injected into guinea pigs.

2.5. Evaluation of conjunctivitis

The magnitude of conjunctival redness and edema was macroscopically judged and expressed as a conjunctivitis intensity score (CIS) according to an arbitrary 5-point graded scale, in which a score of 0 indicates no symptoms; 1, light symptoms; 2, mild symptoms; 3, moderate symptoms; and 4, severe symptoms (Yasuda et al., 1999). Additionally, symptoms falling between scores 0 and 1 were judged as score 0.5.

Scratching frequencies at 0–0.5 and 0.5–1 h after either pollen or chymase challenge were counted. The scratch response was defined as an uninterrupted cluster of rapid hind limb movements that were precisely directed to the ocular surface.

2.6. Treatment with chymase inhibitor and anti-histaminic drug

The chymase inhibitor, ONO-WH-236 (40 and 80 mg/kg) and an anti-histaminic drug, ketotifen (3 mg/kg; Sigma–Aldrich) were orally administered once, 1 h before the 2nd or 3rd pollen challenge. ONO-WH-236 and ketotifen were suspended and dissolved, respectively, in 0.5% methyl cellulose solution before the use. We have reported that inhibition by a classical anti-histaminic drug, pyrilamine, was consistently observed in the 1st–3rd challenges in an allergic conjunctivitis model (Fukushima et al., 2003). In the experiment of chymase-induced conjunctivitis, these compounds were orally administered 1 h before chymase application.

When ONO-WH-236 was once orally administered at a dose of 100 mg/kg in rats, bioavailability was 78%, and the *t*_{1/2} was 3.7 h.

2.7. Measurement of chymase activity and histamine amount in ophthalmic lavage fluid (OLF)

Ophthalmic lavage was performed before, and 10 min, 0.5 h, 1 h, 3 h and 6 h after an antigen challenge. In chymase-induced conjunctivitis, OLF was collected before and 10 min after human chymase administration. Physiologic saline (10 μ l) was applied to the eye using a micropipette without touching the eye. After 2 or 3 forced blinks, OLF was collected. The lavage was repeated 5 times in each eye, and OLF obtained from both eyes was combined.

Following centrifugation, chymase activity in the supernatant was assayed as previously reported (Kirimura et al., 2005). Briefly, OLF was incubated with 2 M KCl and 0.1% Triton X 100-containing 20 mM Tris–HCl (pH 8.5) at 4 °C overnight, followed by incubating in the presence of a substrate, Suc-Ala-Ala-Pro-Phe-4-methylcoumaryl-7-amide (1 mM; Peptide Institute, Osaka, Japan) at 37 °C for 1 h. The enzyme reaction was terminated by the addition of 3% metaphosphoric acid. After centrifugation, 7-amino-4-methylcoumarin (AMC) was measured by fluorophotometric determination (excitation, 390 nm; emission, 460 nm) using a microplate reader (Infinite M200; Tecan Group Ltd., Männedorf, Switzerland). One unit of chymase activity was defined as the amount of enzyme that cleaved 1 μ mol AMC/min. The increase in chymase activity by OLF was inhibited by chymase inhibitors, Suc-Val-Pro-Phe^P(OPh)₂ (10 μ M) and chymostatin (100 μ M) by 99.5% and 41.2%, respectively, indicating that the liberation of AMC reflected chymase activity.

Histamine in the supernatant was assayed by an enzyme immunoassay kit (SPI-Bio, Montigny le Bretonneux, France).

2.8. Statistical analyses

Data are shown as the means $\pm$ S.E. Multiple groups were compared by one-way analysis of variance (ANOVA). When a significant difference was detected, individual group differences were determined by Bonferroni's multiple test. In comparisons between two groups Student's *t*-test was performed. In the experiment of the time-course change in chymase activity, the paired *t*-test was applied. A probability value of *p* < 0.05 was considered significant.

3. Results

3.1. Effect of chymase inhibitor on chymase activity in vitro

IC₅₀ value of ONO-WH-236 for chymase activity was 16 nM, while that for chymotrypsin activity was 4700 nM, which was 300-fold higher than the effect on chymase, suggesting that ONO-WH-236 potently and selectively inhibits chymase activity.

3.2. Effect of chymase inhibitor on allergic conjunctivitis

In the allergic conjunctivitis model of guinea pigs, the pollen challenge produced an immediate increase in CIS, which scores degrees of redness and edema in the conjunctiva, at a peak of 30 min after the challenge (Fig. 1A and B). A single treatment with ONO-WH-236 at 40 and 80 mg/kg dose-dependently inhibited the increase in CIS (Fig. 1A). The inhibitory degree of 80 mg/kg ONO-WH-236 was approximately 50% at 0.5–2 h after the challenge (Fig. 1A). An anti-histaminic drug, ketotifen (3 mg/kg), also inhibited the response by approximately 60% at 0.5–2 h (Fig. 1B).

As shown in Fig. 1C, the pollen challenge induced an itch-associated response, an increase in scratching frequency by the hind paw of sensitized animals, within 1 h after the challenge. Prior to the challenge, scratching behavior was hardly observed. We have reported that a low scratching frequency was induced after the

1st h (Yasuda et al., 1999). The scratching frequency was also markedly reduced by ONO-WH-236 (80 mg/kg), and the magnitude of inhibition was approximately 75% (Fig. 1C). Ketotifen also showed comparable inhibition to ONO-WH-236 (Fig. 1C).

Fig. 1D shows time-course changes in chymase activity in OLF after pollen challenge. The activity was markedly increased 10 min after the challenge with statistical significance, followed by returning to the baseline level at 1 h after the challenge (Fig. 1D).

3.3. Effect of chymase inhibitor on increases in chymase activity and histamine amount in OLF

The increase in chymase activity at 10 min after the challenge was significantly inhibited by ONO-WH-236 (40 and 80 mg/kg) by 50–60% (Fig. 2A). On the other hand, we have reported that histamine was also immediately increased in OLF at 10 min after the challenge (Katayama et al., 2005). Because it has been suggested that chymase anaphylactically released from mast cells was involved in histamine release from the cells (Katunuma et al., 1986; He et al., 1999, 2004), we next examined the effect of a chymase inhibitor, ONO-WH-236, on the increase in histamine amount in OLF. As shown in Fig. 2B, the increase in histamine was markedly inhibited by the chymase inhibitor in a dose-dependent manner, suggesting that histamine release from conjunctival mast cells was mediated by chymase, which was derived from mast cells.

3.4. Chymase-induced conjunctivitis, and effects of chymase inhibitor and anti-histaminic drug on the responses

When human chymase (0.09 and 0.9 μ g/eye) was administered by dropping into the eyes of sensitized guinea pigs, redness was weakly induced 10–30 min after the application (Fig. 3A). In contrast, frequent scratching was relatively clearly induced by the administration of human chymase in a dose-dependent fashion (Fig. 3B). The magnitude of the itch-associated response at 0.9 μ g/eye was almost half of that induced by the pollen challenge (Figs. 3B and 1C). On the other hand, we have experienced that a topical application of the vehicle, PBS did not cause any increases in CIS and scratching frequency in sensitized guinea pigs.

The weakly induced redness in the conjunctiva tended to be suppressed by oral administration of ONO-WH-236 (80 mg/kg) at 10–60 min, although this inhibition was not statistically significant probably because the magnitude of the response was too small (Fig. 3C). Ketotifen did not inhibit inflammation, except for showing a suppressive tendency at 120 min (Fig. 3C). In contrast, the induction of scratching behavior by chymase administration was significantly inhibited by ONO-WH-236 by approximately 50% (Fig. 3D). Interestingly, an anti-histaminic drug, ketotifen, also significantly inhibited chymase-induced scratching (Fig. 3D), suggesting that the response is mediated by released histamine.

Histamine level in OLF 10 min after chymase administration was assayed; however, unexpectedly, histamine concentration in OLF was not significantly increased 10 min after administration: Concentrations of histamine before and 10 min after administration were 4.6 ± 2.2 (*n* = 5) and 11.0 ± 7.2 ng/ml (*n* = 5), respectively.

4. Discussion

Pollen antigen challenge-induced allergic conjunctivitis symptoms, redness and edema in the conjunctiva and itch-associated scratching behavior were effectively suppressed by treatment with a chymase inhibitor, ONO-WH-236. The inhibitory degree at 80 mg/kg was almost comparable to the histamine H₁ receptor antagonist, ketotifen (3 mg/kg). Interestingly, anaphylactic release of histamine in the conjunctiva was markedly suppressed by the

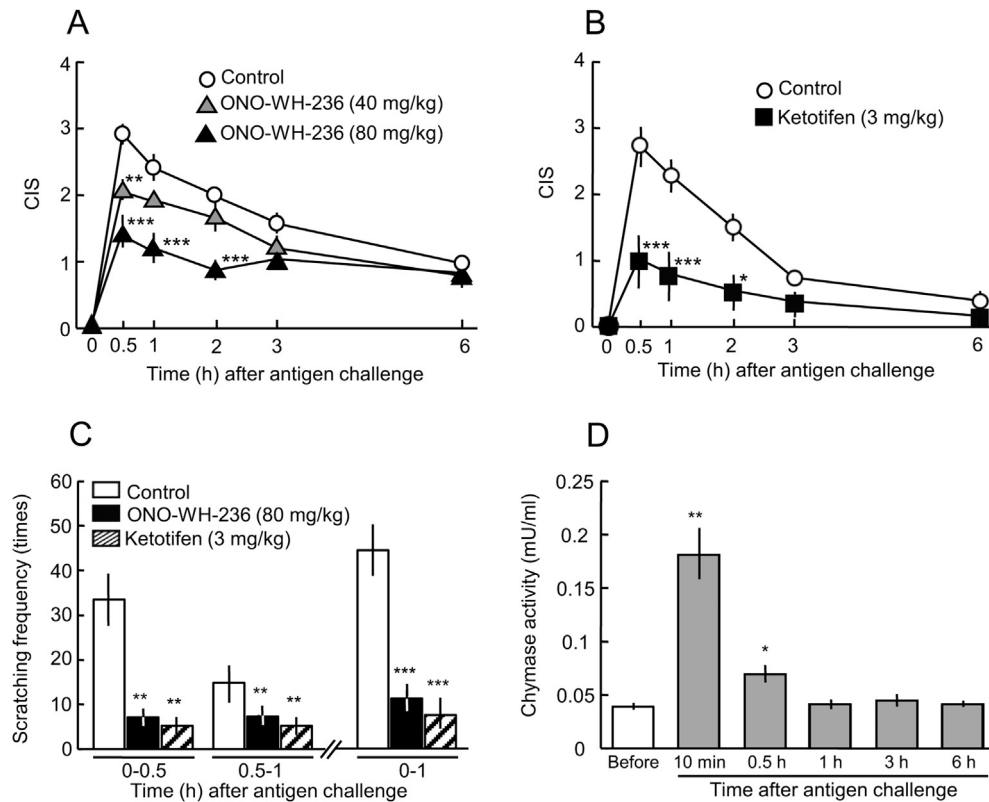


Fig. 1. (A and B) Effects of chymase inhibitor, ONO-WH-236 (A), and anti-histaminic drug, ketotifen (B), on increase in conjunctivitis intensity score (CIS), degrees of redness and edema in conjunctivitis after an antigen challenge with Japanese cedar pollen in sensitized guinea pigs. Guinea pigs were sensitized by two intraperitoneal injections of pollen extract +Al(OH)₃, and then challenged by dropping pollen suspension into both eyes once a week. Effects of ONO-WH-236 and ketotifen were assessed at the 2nd and 3rd challenges, respectively. ONO-WH-236 and ketotifen were orally administered 1 h before the respective pollen challenges. *, ***, $p < 0.05$, 0.001 compared with the control. Each point represents the mean $\pm$ S.E. of 6 (A) and 10 (B) animals. (C) Effects of ONO-WH-236 and ketotifen on increase in scratching frequency after the 3rd antigen challenge with pollen. ONO-WH-236 and ketotifen were orally administered 1 h before the pollen challenge. **, ***, $p < 0.01$, 0.001 compared with control. Each column represents the mean $\pm$ S.E. of 10 animals. (D) Time-course change in chymase activity in ophthalmic lavage fluid (OLF) collected before and after the 2nd pollen challenge. *, **, $p < 0.05$, 0.01 compared with the value before the challenge. Each column represents the mean $\pm$ S.E. of 3 or 6 animals.

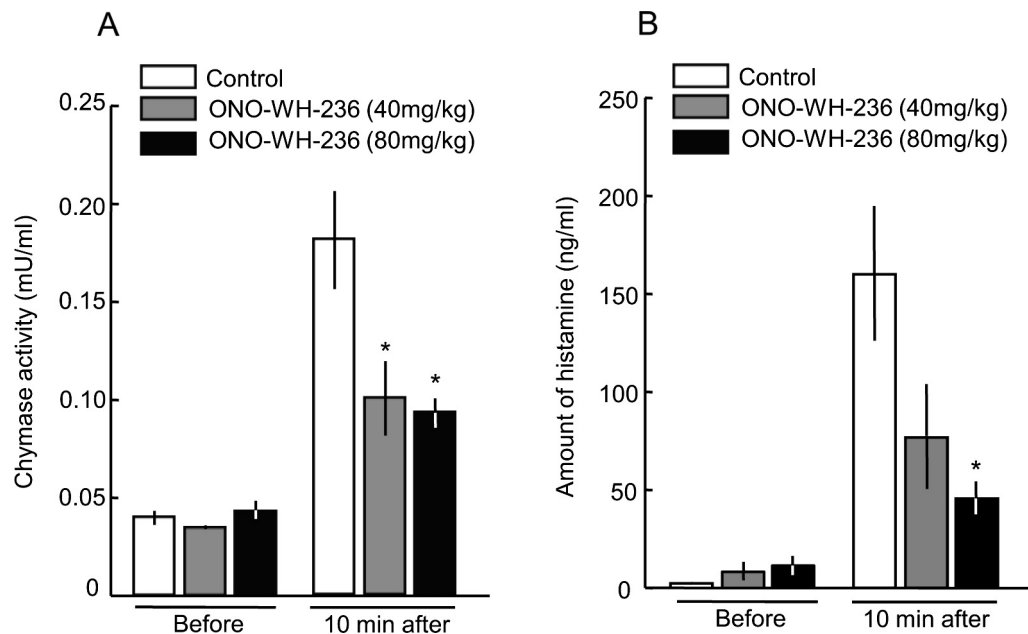


Fig. 2. Effects of chymase inhibitor, ONO-WH-236, on increases in chymase activity (A) and histamine amount (B) in ophthalmic lavage fluid (OLF) collected 10 min after the 2nd challenge with Japanese cedar pollen in sensitized guinea pigs. ONO-WH-236 was orally administered 1 h before the pollen challenge. *, $p < 0.05$ compared with the control. Each column represents the mean $\pm$ S.E. of 6 animals.

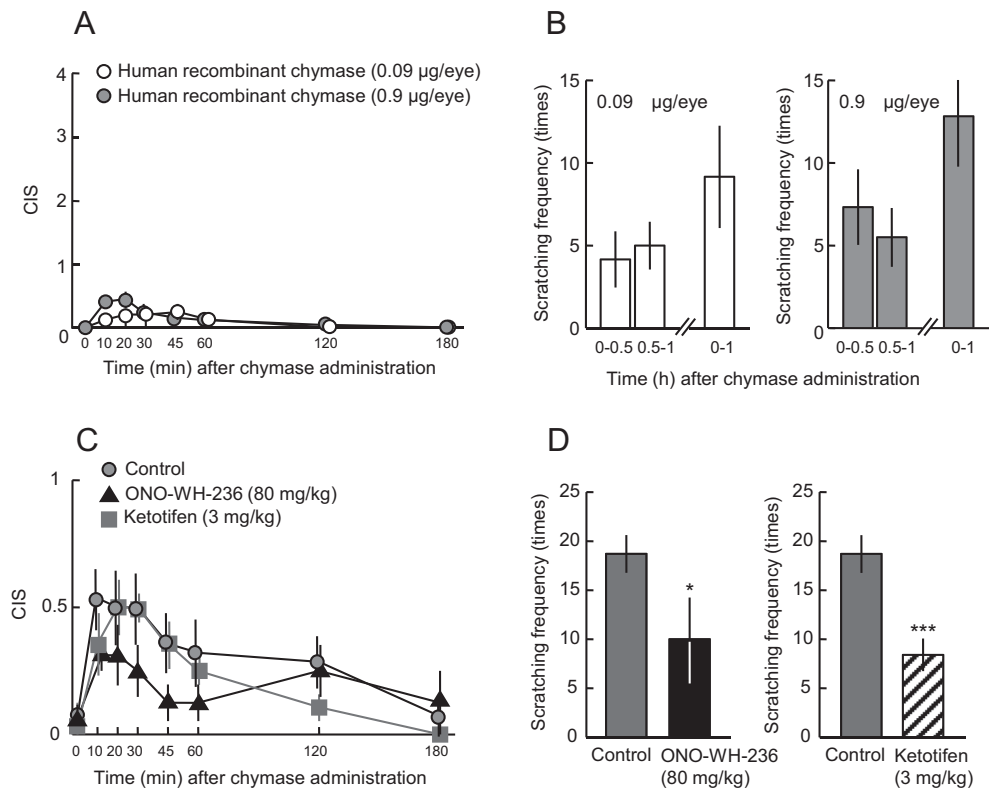


Fig. 3. (A and B) Time-course changes in conjunctivitis intensity score (CIS, A) and scratching frequency (B) after administration of human recombinant chymase (0.09 and 0.9 $\mu\text{g}/\text{eye}$) to sensitized guinea pigs. Human recombinant chymase was dropped into both eyes of the animals. The experiment was performed 7 days after the 3rd pollen challenge. Each point and column represents the mean $\pm$ S.E. of 6 animals. (C and D) Effects of chymase inhibitor, ONO-WH-236, and anti-histaminic drug, ketotifen, on human recombinant chymase (0.9 $\mu\text{g}/\text{eye}$)-induced increases in conjunctivitis intensity score (CIS, C) and scratching frequency (D) in sensitized guinea pigs. ONO-WH-236 and ketotifen were orally administered 1 h before chymase administration. *, ***: $p < 0.05$, 0.001 compared with the control. Each point and column represents the mean $\pm$ S.E. of 7 (control and ketotifen-treated) and 4 (ONO-WH-236-treated) animals.

chymase inhibitor. On the other hand, exogenously applied chymase clearly caused scratching behavior, which was significantly suppressed by the anti-histaminic drug, whereas only weak redness was evoked in the conjunctiva by chymase administration. Thus, there were some controversy in the results between allergen- and chymase-induced conjunctivitis. However, from the results in allergic conjunctivitis, it was strongly suggested that endogenously derived chymase was involved in the formation of ocular allergic symptoms via the release of histamine. This *in vivo* result was consistent with *in vitro* findings, which have reported to date that anaphylactic release of histamine from rat and human mast cells *in vitro* was effectively suppressed by chymase inhibitors (Katunuma et al., 1986; He et al., 1999, 2004).

As mentioned above, exogenously applied chymase could not completely reproduce antigen-induced conjunctivitis: The application of chymase induced only weak redness, which was resistant to anti-histamine treatment. Furthermore, histamine was not clearly released by exogenous chymase in the conjunctiva. This controversy may be simply due to that a large molecule, chymase could not act on mast cells, when exogenously applied. However, the failure of exogenous chymase to induce responses were not surprising because, similar to the present result, it was demonstrated that, unlike tryptase, exogenous chymase did not induce histamine release from human mast cells even though anaphylactic release of histamine was suppressed by chymase inhibitors (He et al., 1999, 2001). Additionally, another paper reported that microvascular leakage induced in the skin of guinea pigs by human chymase was unaffected by anti-histamine treatment (He and Walls, 1998). Nevertheless, studies of chymase inhibitors

(Katunuma et al., 1986; He et al., 1999, 2004) clearly suggest that chymase may play roles in anaphylactic histamine release from mast cells. The controversial function of chymase between allergen- and chymase-induced conjunctivitis in the present experiments may be due to the nature of the substrate to be cleaved by chymase, and its precise location, as suggested by He et al. (1999). Endogenously derived chymase may cleave a substrate within mast cells, leading to histamine release. Alternatively, anaphylactic stimulation may activate mast cells to expose substrates to be cleaved by chymase within and/or on the surface of the cells. Supporting evidence of this consideration is that IgE-dependent activation of rat peritoneal mast cells was associated with increased intracellular chymotryptic activity (Khiav and Pearce, 1998). It has been reported that a variety of pro-inflammatory molecules, such as pro-IL-1 β (Mizutani et al., 1991), pro-IL-18 (Omoto et al., 2006), and CCR1 ligands (Berahovich et al., 2005), are cleaved by chymase. Analyses of target molecules (substrates) of chymase in mast cells can be useful to elucidate the mechanisms underlying chymase-induced pro-inflammatory actions.

Antigen-induced vasodilatation and plasma leakage, manifested by redness and edema, in the conjunctiva were suppressed by the chymase inhibitor and anti-histaminic drug by a similar degree. Thus, the function of chymase to induce vascular responses may be dependent on histamine. However, inhibition by the compounds was not complete (50–60%), suggesting that mediators other than histamine and chymase participate in the induction of an immediate allergic response. We have reported that a non-selective nitric oxide synthase (NOS) inhibitor, *N*^ω-nitro-L-arginine methyl ester, almost completely suppressed the pollen-induced increase in CIS

(Fukushima et al., 2003), suggesting that nitric oxide (NO) plays major roles in the vascular response. Histamine may be one of the mediators activating NOS. Additionally, NO production must be mediated by other molecules that have not been identified. In contrast, antigen-induced scratching frequency was largely dependent on histamine, which might be released by endogenous chymase because the antigen-induced response was nearly completely suppressed by anti-histamine treatment.

On the other hand, exogenously applied chymase clearly induced scratching behavior, although the protease caused only weak redness in the conjunctiva. In addition, degree of the inhibition on the exogenous chymase-induced scratching by ONO-WH-236 was not complete even though the antigen-induced scratching was almost completely dependent on chymase. Regarding these differences, we can speculate that some substrates susceptible to chymase may exist on the cellular surface of the sensory nerve endings of the conjunctiva, but not on vascular cells. One of the candidate molecules is protease-activated receptor (PAR)-2, which has been reported to be activated by another protease, tryptase (Corvera et al., 1997; Mirza et al., 1997; Molino et al., 1997). A couple of papers have suggested that chymase also activates PAR-2 on certain cells (Sharma et al., 2007; Groschwitz et al., 2013). Regarding the relationship between PAR-2 and itch, Ui et al. (2006) reported that intradermal injection of tryptase (0.1 ng/site) clearly elicited a PAR-2-mediated scratching response, while chymase (50 ng/site) did not exert a scratching response in mice. The lower dose of chymase used in this study was 90 ng/eye, which was not largely different from that in this paper (Ui et al., 2006), and clearly increased scratching frequency in our study of guinea pigs. There may be species or tissue differences in chymase-induced itching. In addition, even though only a small amount of histamine was released by exogenous chymase applied to the eyes, histamine-dependent scratching behavior, but not redness, was evoked in the conjunctiva. This may imply that sensory nerve endings are more susceptible to histamine via H₁ receptor activation than the vascular cells that are involved in the induction of redness and plasma leakage.

In conclusion, it was suggested that chymase was released from mast cells after an antigen challenge, followed by the induction of conjunctivitis symptoms through histamine release from mast cells. Thus, chymase could be a potential target for pharmacotherapy for allergic conjunctivitis.

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