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Original Paper

Magnesium and Zinc Dose-Dependently Stabilize Rat Peritoneal Mast Cells and Enhance the Effects of Adrenaline

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Key Words
Adrenaline • Magnesium • Mast cell-stabilizing properties • Transient receptor potential cation channel subfamily M member 7 (TRPM7) • Zinc

Abstract
Background/Aims: Magnesium and zinc are vital trace elements found in numerous foods and dietary supplements. In addition to their antioxidant, anticancer, antibacterial, and anti-inflammatory effects, clinical research has suggested that they possess anti-allergic properties. ***Materials:*** Using differential-interference contrast (DIC) microscopy, we examined the effects of magnesium chloride ($MgCl_2$) and zinc chloride ($ZnCl_2$) on rat peritoneal mast cell degranulation. We also examined their effects in conjunction with adrenaline, the first-choice drug for anaphylaxis treatment. ***Results:*** Both $MgCl_2$ and $ZnCl_2$ reduced the number of degranulating mast cells in a dose-dependent manner. $MgCl_2$ significantly decreased the number of degranulating mast cells at concentrations of 50 mM or higher, whereas $ZnCl_2$ achieved similar effects at much lower concentrations of 25 μM or more. These levels of $MgCl_2$ or $ZnCl_2$ enhanced the inhibitory effects of 1 mM adrenaline on mast cell degranulation. Additionally, pharmacological inhibition of the transient receptor potential cation channel subfamily M member 7 (TRPM7) by NS8593 reduced the number of degranulating mast cells in a dose-dependent manner. ***Conclusion:*** This study is the first to provide *in vitro* evidence that magnesium and zinc stabilize mast cells in a dose-dependent manner and also enhance the effects of adrenaline. TRPM7, which has higher permeability to zinc ions than to magnesium ions, may contribute to the stronger mast cell-stabilizing properties of zinc.

Introduction

Previous studies have revealed health-promoting properties of magnesium and zinc, including antioxidant, anti-cancer, antibacterial, and anti-inflammatory effects [1, 2]. Recently, in humans, magnesium and zinc have been reported to alleviate the symptoms

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of allergic rhinitis and allergic skin diseases [3-5], suggesting their potential as anti-allergic agents and opening new pharmacological possibilities. These anti-allergic effects have also been observed in animal models of allergic diseases such as atopic dermatitis, asthma, and anaphylaxis [6-8]. Previous studies have revealed the molecular mechanisms underlying the anti-inflammatory properties of magnesium and zinc using isolated lymphocytes or macrophages [1, 9]. In humans, deficiencies in these trace elements were found to be associated with the progression of inflammatory diseases [1, 9]. However, the exact mechanisms underlying the anti-allergic properties of magnesium and zinc remain largely unknown. During allergic reactions, mast cells have been shown to release secretory granules containing chemical mediators, such as histamine, serotonin, leukotrienes, and prostaglandins through exocytosis [10]. Using mast cells isolated from humans or animals, previous *in vitro* studies determined the anti-allergic properties of magnesium and zinc by showing their inhibitory effects on the release of histamine [11-13]. However, these past studies did not show the direct effects of magnesium or zinc on the process of exocytosis, nor did they show the effects of these trace elements at doses higher than physiological concentrations. Most anti-allergic medications work by blocking histamine H1 receptors in peripheral tissues [14]. However, some drugs or natural compounds exhibit strong anti-allergic effects by directly inhibiting exocytosis, thereby stabilizing mast cells [15]. Employing the standard patch-clamp whole-cell recording technique in rat peritoneal mast cells [16, 17], previous studies have shown that exocytosis is correlated with a gradual increase in the membrane [18-20]. Applying these techniques in our previous study, we showed through continuous monitoring of exocytosis in mast cells that substances such as adrenaline, macrolide antibiotics, corticosteroids, antihypertensives, and anti-allergic drugs have mast cell-stabilizing properties [21-26]. Recently, we demonstrated that food components such as caffeine, catechin, vitamins, and lemon constituents stabilize mast cells [27-29]. In this study, we aimed to assess the anti-allergic properties of magnesium and zinc and to uncover the physiological mechanisms involved by directly examining their impact on rat peritoneal mast cell degranulation. Here, we provide *in vitro* evidence for the first time that magnesium and zinc stabilize mast cells in a dose-dependent manner and enhance the effects of adrenaline. Transient receptor potential cation channel subfamily M member 7 (TRPM7), which is expressed in mast cells [30, 31] and is more permeable to zinc ions (Zn^{2+}) than to magnesium ions (Mg^{2+}) [32, 33], may play a role in the stronger mast cell-stabilizing properties of zinc.

Materials and Methods

Cell Sources and Preparation

Male Wistar rats, which are outbred albino rats and are currently the most commonly used rats in laboratory research [34], were acquired from The Jackson Laboratory Japan, Inc. (Yokohama, Japan) at a minimum age of 25 weeks. Historically, Wistar rats have been frequently used to isolate mast cells [35, 36]. Mast cells extracted from rats within this age bracket were sufficiently viable to undergo exocytosis when exposed to external pharmacological stimuli [21-25, 28, 29, 37]. Due to variations in sex hormones [38], female mast cells are generally more hypersensitive than male mast cells. Consequently, only male rats were used in the experiments performed in this study. The rats were anesthetized using isoflurane and euthanized by cervical dislocation in accordance with the euthanasia guidelines for adult laboratory rodents [39]. The Animal Care and Use Committee of Miyagi University approved the animal protocols (No. 2025-02). As previously outlined [21-28, 40], the rat peritoneum was rinsed with a standard external (bathing) solution, which included: NaCl, 145 mM; KCl, 4.0 mM; $CaCl_2$, 1.0 mM; $MgCl_2$, 2.0 mM; HEPES, 5.0 mM; bovine serum albumin, 0.01 % (pH 7.2 adjusted with NaOH); and mast cells were isolated from the peritoneal cavity. The isolated mast cells were kept in the external solution at room temperature (22-24°C) for about 8 hours until they were used. The mast cell suspension, approximately 200/μL, was distributed in a chamber positioned at the head of an inverted microscope (Nikon, Tokyo, Japan). In the peritoneal cavity of rats, mast cells account for approximately 25% of all the cells [41, 42]. They are easily identifiable from

other cell types owing to their distinctive intracellular secretory granules [21-29, 40, 43], which are smaller and more numerous than basophils [44]. The viability of mast cells was assessed based on their ability to release secretory granules in response to external stimuli (Fig. 1Ab vs. a) and their morphological integrity under differential-interference contrast (DIC) microscopy, as previously demonstrated [45, 46].

Quantification of Mast Cell Degranulation

$MgCl_2$ and $ZnCl_2$, obtained from Wako Pure Chemical Ind. (Osaka, Japan), were separately dissolved in the external solution at final concentrations of 10, 25, 50, and 100 mM and 10, 25, 50, and 100 μM, respectively. In previous *in vitro* studies using cultured or primary human blood cells, concentrations as high as 50 -100 mM magnesium or 50 - 100 μM zinc were required to exert anti-inflammatory properties [47-50]. Therefore, we used $MgCl_2$ or $ZnCl_2$ in this study, starting from these concentrations. $MgCl_2$ and $ZnCl_2$ were also dissolved in an external solution containing 1 mM adrenaline (Daiichi Sankyo, Inc., Tokyo, Japan) at final concentrations of 50, 100 mM and 50, 100 μM. NS8593 was purchased from Cayman Chemical (Ann Arbor, MI, USA) and dissolved to final concentrations of 10, 25, 50, and 100 μM. After incubating mast cells with these solutions or the external solution alone for 10 min, exocytosis was externally induced using compound 48/80 (Sigma-Aldrich Co., St. Louis, MO, USA; final concentration, 10 μg/mL) [21-29, 40]. We utilized rat-derived mast cells in our experiments as they are more responsive to compound 48/80 than those isolated from the mouse peritoneal cavity [51]. Bright-field images were captured from randomly selected 0.1-mm^2 fields of view (10 views from each condition), as previously described [21-29, 40, 43]. We counted the number of degranulated mast cells (defined as cells surrounded by more than eight granules outside the cell membrane) and calculated their ratio to the total number of mast cells.

Immunohistochemistry

Parietal or visceral peritoneal walls were removed from the rats for histological examination, as previously described [52]. The 3-μM paraffin sections of 4% paraformaldehyde-fixed peritoneal walls were placed in citrate-buffered solution (pH 6.0) and then boiled for 30 min for antigen retrieval. Endogenous peroxidase was blocked with 3% hydrogen peroxide, and nonspecific binding was blocked with 10% BSA. Mouse anti-TRPM7 (1:50; StressMarq Biosciences Inc., Vitoria, Canada) was used as the primary antibody. Diaminobenzidine substrate (Sigma Chemical Co., St. Louis, MO, USA) was used for the color reaction. Secondary antibodies alone were found to be consistently negative in all of the sections. Toluidine blue staining was additionally performed by immersing sections in 0.1% toluidine blue (Muto Pure Chemical Co., Tokyo, Japan) for 30 min at room temperature_ENREF_18. Mast cells were identified based on their characteristic metachromasia [52].

Statistical Analysis

Data were analyzed using Microsoft Excel (Microsoft Corporation, Redmond, WA., USA) and reported as means ± SEM. Statistical significance was assessed using ANOVA. Statistical significance was set at $p < 0.05$.

Results

Effects of magnesium on mast cell degranulation

Mast cells exposed only to the external solution or to lower concentrations of $MgCl_2$ (10 and 25 mM) exhibited numerous surface wrinkles and secretory granule release via exocytosis (Fig. 1Ab-d vs. a). In contrast, when mast cells were incubated with higher $MgCl_2$ concentrations (50 and 100 mM), signs of exocytosis were partially or entirely absent (Fig. 1Ae, f). Lower $MgCl_2$ concentrations (10 and 25 mM) did not alter the number of degranulating mast cells (Fig. 1B). However, 50 mM $MgCl_2$ significantly reduced the number of degranulating mast cells (control, 94.0 ± 3.37 % vs. 50 mM $MgCl_2$, 72.5 ± 4.53 %; n=10, P<0.05), and 100 mM $MgCl_2$ more markedly decreased the number of degranulating cells (21.0 ± 4.88 %; n=10, P<0.05; Fig. 1B). These findings therefore suggest that $MgCl_2$ inhibits exocytosis in a dose-dependent manner, thereby stabilizing mast cells.

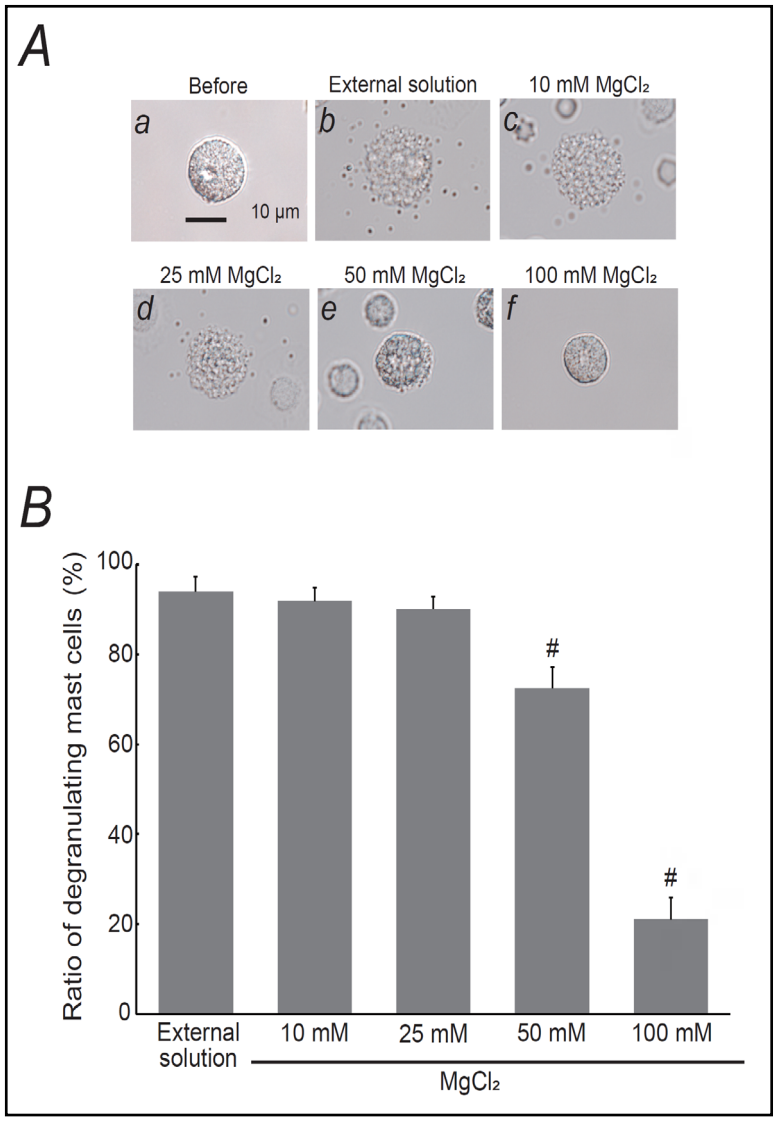

Fig. 1. Effects of magnesium chloride ($MgCl_2$) on mast cell degranulation. A: Differential-interference contrast (DIC) microscopic images were taken before (a) and after exocytosis was externally induced by compound 48/80 in mast cells incubated in the external solutions containing no substance (b) or 10 mM $MgCl_2$ (c), 25 mM $MgCl_2$ (d), 50 mM $MgCl_2$ (e), and 100 mM $MgCl_2$ (f). B: After the mast cells were incubated in the external solutions containing no substance or different concentrations of $MgCl_2$, exocytosis was induced by compound 48/80. From a single rat, several samples of mast cell suspension were obtained from the peritoneal cavity. The aliquot of the sample was spread in a chamber placed at the head stage of an inverted microscope. Then bright-field images were obtained from randomly chosen 0.1-mm^2 fields of view, in which 30-40 mast cells were evenly observed per field. The degranulating mast cells were expressed as the average percentages of the total mast cells in the 10 bright fields. # $p<0.05$ vs. incubation in the external solution alone. Values were presented as the means ± SEM. Differences were analyzed using ANOVA followed by Dunnett's t-test. The experiments were repeated at least three times using three different rats to confirm the reproducibility of the data.

Effects of zinc on mast cell degranulation

Similar to the results obtained with $MgCl_2$ (Fig. 1), a lower concentration of $ZnCl_2$ (10 μM) did not affect mast cell degranulation (Fig. 2Ac vs. b), with degranulating cell numbers nearly matching those in the external solution alone (Fig. 2B). However, 25 μM $ZnCl_2$ partially inhibited exocytosis (Fig. 2Ad) and significantly reduced the number of degranulating mast cells (control, 98.5 ± 0.77 % vs. 25 μM $ZnCl_2$, 85.0 ± 1.28 %; n=10, P<0.05; Fig. 2B). Of note, 50 and 100 μM $ZnCl_2$ almost completely halted exocytosis (Fig. 2Ae, f) and further reduced the number of degranulating mast cells (50 μM $ZnCl_2$, 37.3 ± 2.31 %; 100 μM $ZnCl_2$, 32.6 ± 1.93 %; n=10, P<0.05; Fig. 2B). These results indicate that, similar to $MgCl_2$, $ZnCl_2$ inhibits exocytosis in a dose-dependent manner and stabilizes mast cells, but requires much lower doses than $MgCl_2$ in order to achieve these effects (Fig. 2B vs. 1B).

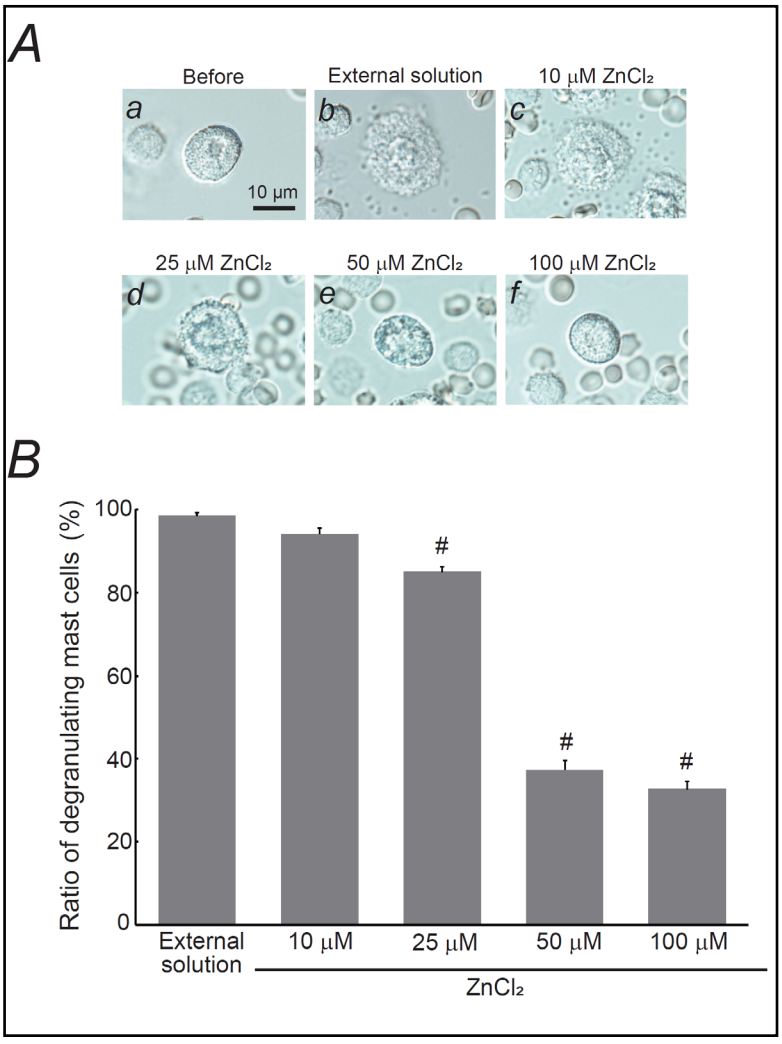

Fig. 2. Effects of zinc chloride ($ZnCl_2$) on mast cell degranulation. A: Differential-interference contrast (DIC) microscopic images were taken before (a) and after exocytosis was externally induced by compound 48/80 in mast cells incubated in the external solutions containing no substance (b) or 10 μM $ZnCl_2$ (c), 25 μM $ZnCl_2$ (d), 50 μM $ZnCl_2$ (e), and 100 μM $ZnCl_2$ (f). B: After the mast cells were incubated in the external solutions containing no substance or different concentrations of $ZnCl_2$, exocytosis was induced by compound 48/80. From a single rat, several samples of mast cell suspension were obtained from the peritoneal cavity. The aliquot of the sample was spread in a chamber placed at the head stage of an inverted microscope. Then bright-field images were obtained from randomly chosen 0.1-mm^2 fields of view, in which 30-40 mast cells were evenly observed per field. The degranulating mast cells were expressed as the average percentages of the total mast cells in the 10 bright fields. # $p < 0.05$ vs. incubation in the external solution alone. Values were presented as the means ± SEM. Differences were analyzed using ANOVA followed by Dunnett's t-test. The experiments were repeated at least three times using three different rats to confirm the reproducibility of the data.

Combined effects of magnesium or zinc with adrenaline on mast cell degranulation

In our previous study, adrenaline, a first-line drug for anaphylaxis [53], was found to inhibit exocytosis in mast cells in a dose-dependent manner [25]. However, even at the highest concentration of 1 mM, the suppressive effect of adrenaline was found to be insufficient [25]. Given that higher concentrations of $MgCl_2$ and $ZnCl_2$ significantly suppressed mast cell degranulation (Fig. 1 and 2), we have examined their combined effects with adrenaline (Fig. 3). Consistent with our previous findings [25], 1 mM adrenaline significantly reduced the number of degranulated mast cells (Fig. 3B and C). Notably, the presence of $MgCl_2$ (50 and 100 mM) or $ZnCl_2$ (50 and 100 μM), which significantly decreased the number of degranulating mast cells (Fig. 1B and 2B), effectively halted exocytosis process in these cells (Fig. 3Ac-f vs. b). Regarding the numbers of degranulating mast cells, there was a substantial decrease when compared to those treated with 1 mM adrenaline alone (1 mM adrenaline, 53.3 ± 1.24 % vs. 1 mM adrenaline + 50 mM $MgCl_2$, 23.7 ± 3.86 %, n=10, P<0.05; 1 mM adrenaline + 100

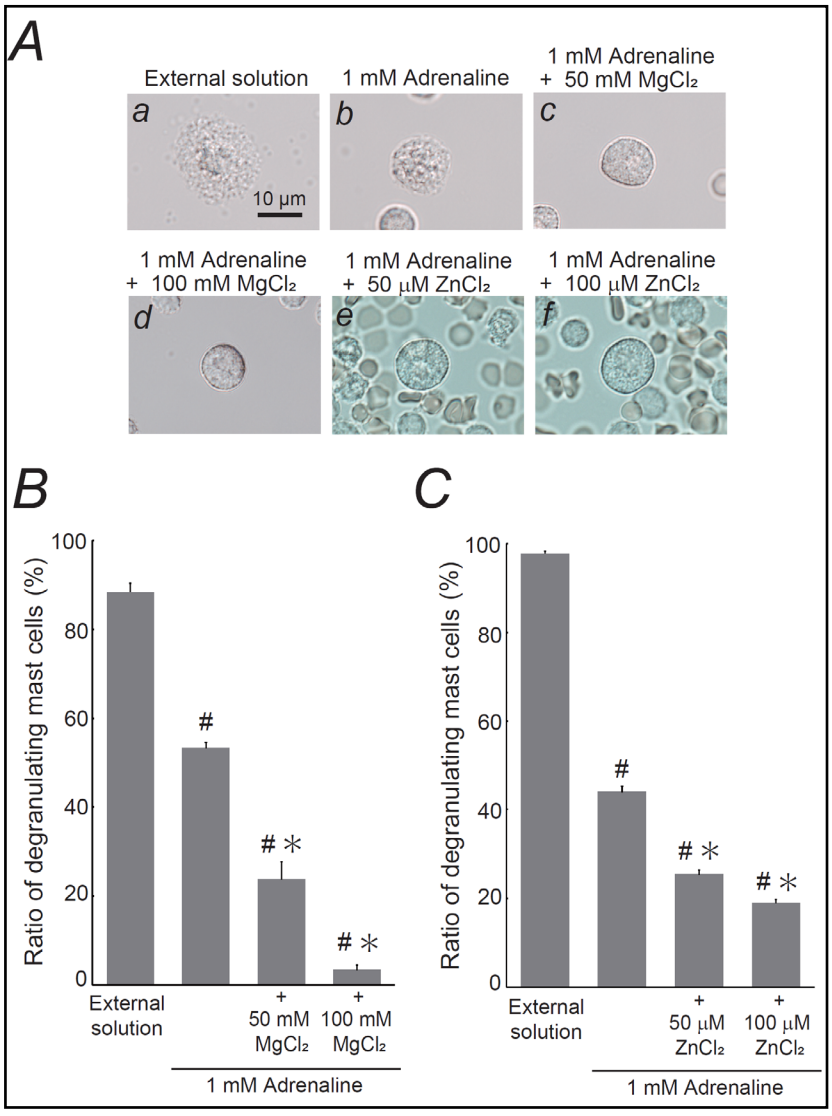

Fig. 3. Effects of magnesium chloride ($MgCl_2$) or zinc chloride ($ZnCl_2$) on adrenaline-induced inhibition of mast cell degranulation. A: Differential-interference contrast (DIC) microscopic images were taken after exocytosis was externally induced by compound 48/80 in mast cells incubated in the external solutions containing no substances (a), 1 mM adrenaline alone (b), 1 mM adrenaline in the presence of 50 mM $MgCl_2$ (c), 1 mM adrenaline in the presence of 100 mM $MgCl_2$ (d), 1 mM adrenaline in the presence of 50 μM $ZnCl_2$ (e), and 1 mM adrenaline in the presence of 100 μM $ZnCl_2$ (f). B: After exocytosis was induced in mast cells incubated in the external solutions containing no substance, 1 mM adrenaline alone, 1 mM adrenaline in the presence of 50 or 100 mM $MgCl_2$, the numbers of degranulating mast cells were expressed as percentages of the total mast cell numbers in selected bright fields. C: After exocytosis was induced in mast cells incubated in the external solutions containing no substance, 1 mM adrenaline alone, 1 mM adrenaline in the presence of 50 or 100 μM $ZnCl_2$, the numbers of degranulating mast cells were expressed as percentages of the total mast cell numbers in selected bright fields. From a single rat, several samples of mast cell suspension were obtained from the peritoneal cavity. The aliquot of the sample was spread in a chamber placed at the head stage of an inverted microscope. Then bright-field images were obtained from randomly chosen 0.1-mm^2 fields of view, in which 30-40 mast cells were evenly observed per field. The degranulating mast cells were expressed as the average percentages of the total mast cells in the 10 bright fields. # $p < 0.05$ vs. incubation in the external solution alone. *$p < 0.05$ vs. incubation in the external solution containing 1 mM adrenaline. Values were presented as the means ± SEM. Differences were analyzed by ANOVA followed by Dunnett's t-test. The experiments were repeated at least three times using three different rats to confirm the reproducibility of the data.

mM $MgCl_2$, 3.34 ± 1.08 %, n=10, P<0.05; Fig. 3B) (1 mM adrenaline, 43.9 ± 1.31 % vs. 1 mM adrenaline + 50 μM $ZnCl_2$, 25.4 ± 0.94 %, n=10, P<0.05; 1 mM adrenaline + 100 μM $ZnCl_2$, 18.9 ± 0.80 %, n=10, P<0.05; Fig. 3C). These observations indicate that the inhibitory effects of adrenaline on exocytosis were enhanced, with higher concentrations of $MgCl_2$ or $ZnCl_2$ additively potentiating the mast cell-stabilizing properties of adrenaline.

Role of TRPM7 in mast cell degranulation

TRPM7 is a channel for divalent cations that also allow the passage of Mg^{2+} and Zn^{2+} [32, 33]. Since TRPM7 is present in mast cells derived from humans and mice [30, 31], we examined its expression in rat peritoneal mast cells (Fig. 4A). In tissue sections of the rat peritoneum, mast cells were identified based on their distinct metachromasia when stained with toluidine blue (Fig. 4A, arrows). Consistent with previous findings in murine mast cells [31], immunohistochemistry for TRPM7 showed positive expression in both the plasma membrane, cytoplasm, and adipose tissue [54]. To reveal the involvement of TRPM7 in mast cell degranulation, exocytosis was triggered in the presence of its potent inhibitor, NS8593 [55, 56] (Fig. 4B and C). Mast cells treated with 10 μM NS8593 showed no change in the number of degranulated cells (Fig. 4C). However, 25 μM NS8593 was found to have significantly reduced the number of degranulating mast cells (control, 96.3 ± 0.80 % vs.

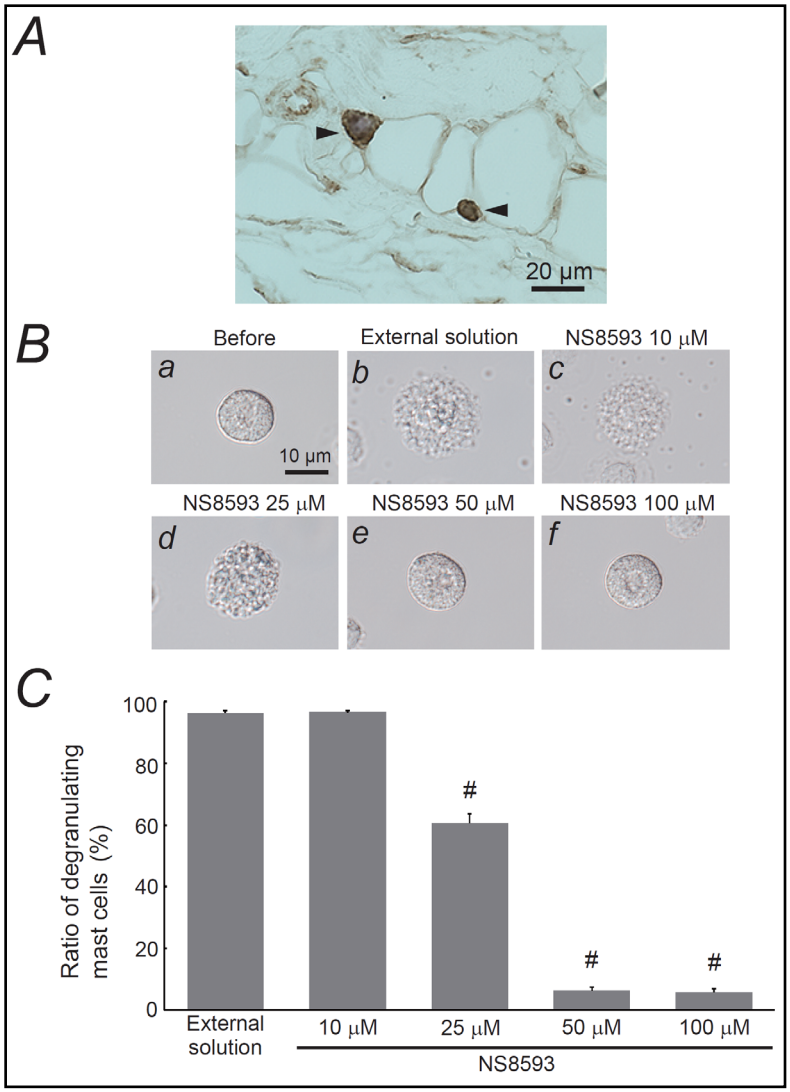

Fig. 4. Expression of transient receptor potential cation channel subfamily M member 7 (TRPM7) in mast cells and its involvement in mast cell degranulation. A: Immunohistochemistry using an antibody for anti-TRPM7 (brown) in rat peritoneal mast cells and adipose tissue, co-stained with 0.1% toluidine blue. Magnification X 60. B: Differential-interference contrast (DIC) microscopic images were taken before (a) and after exocytosis was externally induced by compound 48/80 in mast cells incubated in the external solutions containing no substance (b) or 10 μM NS8593 (c), 25 μM NS8593 (d), 50 μM NS8593 (e), and 100 μM NS8593 (f). C: After the mast cells were incubated in the external solutions containing no substance or different concentrations of NS8593, exocytosis was induced by compound 48/80. From a single rat, several samples of mast cell suspension were obtained from the peritoneal cavity. The aliquot of the sample was spread in a chamber placed at the head stage of an inverted microscope. Then bright-field images were obtained from randomly chosen 0.1-mm^2 fields of view, in which 30-40 mast cells were evenly observed per field. The degranulating mast cells were expressed as the average percentages of the total mast cells in the 10 bright fields. # $p < 0.05$ vs. incubation in the external solution alone. Values were presented as the means ± SEM. Differences were analyzed using ANOVA followed by Dunnett's t-test. The experiments were repeated at least three times using three different rats to confirm the reproducibility of the data.

25 μM NS8593, 60.7 ± 2.89 %; *n*=10, *P*<0.05; Fig. 4C), and even greater reductions were observed with 50 or 100 μM NS8593 (50 μM NS8593, 6.29 ± 1.13 %; *n*=10, *P*<0.05; 100 μM NS8593, 5.72 ± 1.20 %; *n*=10, *P*<0.05; Fig. 4C). These results suggest that the pharmacological inhibition of TRPM7 suppresses exocytosis in a dose-dependent manner, highlighting the crucial role of TRPM7 in mast cell degranulation.

Discussion

In addition to chemical mediators, such as histamine, serotonin, leukotrienes, and prostaglandins, mast cells release various cytokines and growth factors via exocytosis [10]. Therefore, to accurately assess the mast cell-stabilizing properties of drugs or substances, it is essential to directly observe exocytosis rather than indirectly measure the levels of chemical mediators released [18-22, 24, 43, 57]. In this study, we meticulously observed the entire exocytosis process under a microscope, defining it as the proportion of degranulating mast cells, to focus on the release of all chemical mediators [21-26, 29]. This method allowed us to demonstrate *in vitro* that adrenaline, macrolide antibiotics (clarithromycin), corticosteroids (dexamethasone and hydrocortisone), anti-hypertensives (prazosin), and anti-allergic drugs (tranilast, ketotifen, olopatadine, and cetirizine) possess mast cell-stabilizing properties [21-26]. Furthermore, we recently showed that food components such as caffeine, catechins, vitamins, and elements from lemon juice or peel (citric acid, hesperetin, and eriodictyol) stabilize mast cells and exert synergistic effects when combined [27-29]. In this study, using the same methodology, we provided direct evidence that essential trace elements such as magnesium and zinc inhibit exocytosis in a dose-dependent manner, thereby exhibiting mast cell-stabilizing properties (Fig. 1 and 2). In order to determine the effects of these trace elements, we examined the direct effects of $MgCl_2$ and $ZnCl_2$ on mast cell degranulation in the present study. However, as an electrolyte, chloride ions (Cl^-) may affect the cell membrane potential and thus indirectly inhibit Ca^{2+} influx into mast cells [58]. Additionally, studies revealed that the pharmacological blockade of Cl^- channels in mast cells modulate exocytosis [59, 60]. Therefore, caution should be exercised when interpreting these results.

Adrenaline is typically administered as a first-line treatment for anaphylaxis [53]. However, in our previous research, the ability of adrenaline to suppress mast cell degranulation was inadequate at the highest concentration of 1 mM [25]. Additionally, there have been cases of adrenaline-resistant refractory anaphylaxis in which patients did not respond well to adrenaline treatment [61-63]. Since adrenaline inhibits mast cell degranulation through the β_2-adrenergic pathway [25], patients regularly taking β-adrenergic receptor blockers often show resistance to adrenaline [61]. Moreover, the use of perioperative drugs, such as muscle relaxants, certain antibiotics, and radiocontrast media, can increase the risk of adrenaline-resistant anaphylaxis, as these agents directly stimulate mast cells via the Mas-related G-protein coupled receptor member X2 (MRGPRX2), increasing the severity of the condition [62, 63]. In the present study, higher concentrations of $MgCl_2$ and $ZnCl_2$ enhanced the mast cell-stabilizing properties of adrenaline (Fig. 3). Thus, these trace elements may be beneficial in regard to augmenting the effects of adrenaline in cases of adrenaline-resistant refractory anaphylaxis.

In addition to their role in allergic reactions, mast cells are involved in the development of fibrosis in organs such as the lungs, liver, kidneys, and skin [64-66]. Under conditions, such as chronic inflammation, these cells release factors that activate fibroblasts, thereby worsening organ fibrosis [10]. Consequently, treatments that stabilize mast cells or inhibit chemokines that directly reduce mast cell activity have been shown to be effective against organ fibrosis [67-70]. Our previous study indicated that tranilast, a potent mast cell stabilizer, slowed the progression of peritoneal fibrosis in a rat model of chronic uremia [22]. In the current study, magnesium and zinc were identified as effective mast cell stabilizers (Fig. 1 and 2), suggesting their potential for treating or preventing organ fibrosis. Recent studies in humans and animals have linked magnesium and zinc deficiencies to the progression

of organ fibrosis, including liver cirrhosis, renal fibrosis, and pulmonary fibrosis, and have shown that these trace elements can improve these conditions [71-75].

In this study, both $MgCl_2$ and $ZnCl_2$ demonstrated dose-dependent mast cell-stabilizing properties (Fig. 1 and 2) and they were shown to have enhanced the effects of adrenaline (Fig. 3). Notably, $ZnCl_2$ appeared to be more potent than $MgCl_2$ because much lower doses were required to achieve similar effects (Fig. 2 and 3). Our previous patch-clamp study showed that ethylene glycol tetra-acetic acid (EGTA), which chelates calcium ions (Ca^{2+}) and blocks their intracellular transport, completely inhibits exocytosis [22]. Thus, consistent with earlier findings [16, 76], an increase in the intracellular Ca^{2+} concentration is considered the main trigger for mast cell exocytosis. Our results suggest that TRPM7, which is expressed in mast cells, plays a crucial role in mast cell degranulation (Fig. 4). TRPM7 facilitates Ca^{2+} entry into cells, contributing to an increase in the intracellular Ca^{2+} concentration in mast cells [30] (Fig. 5). As a channel for divalent cations, TRPM7 is also permeable to Mg^{2+} and Zn^{2+}, which are thought to hinder Ca^{2+} entry into mast cells, thereby suppressing exocytosis. Importantly, since TRPM7 is more permeable to Zn^{2+} than to Mg^{2+} [32, 77], Zn^{2+} is believed to more effectively block Ca^{2+} entry than Mg^{2+} (Fig. 5), resulting in a stronger mast cell-stabilizing property of zinc when compared to magnesium. Additionally, once inside the cell, zinc modulates the p38 mitogen-activated protein kinase (p38MAPK) or nuclear factor-kappa B (NF-κB) signaling pathways, which are necessary for mast cell degranulation [78, 79]. This may provide zinc with additional mast cell-stabilizing properties.

Conclusion

This study provides novel *in vitro* evidence for the first time that magnesium and zinc dose-dependently stabilize mast cells and additively potentiate the effects of adrenaline. TRPM7, which has higher permeability to Zn^{2+} than to Mg^{2+}, may contribute to the stronger mast cell-stabilizing properties of zinc.

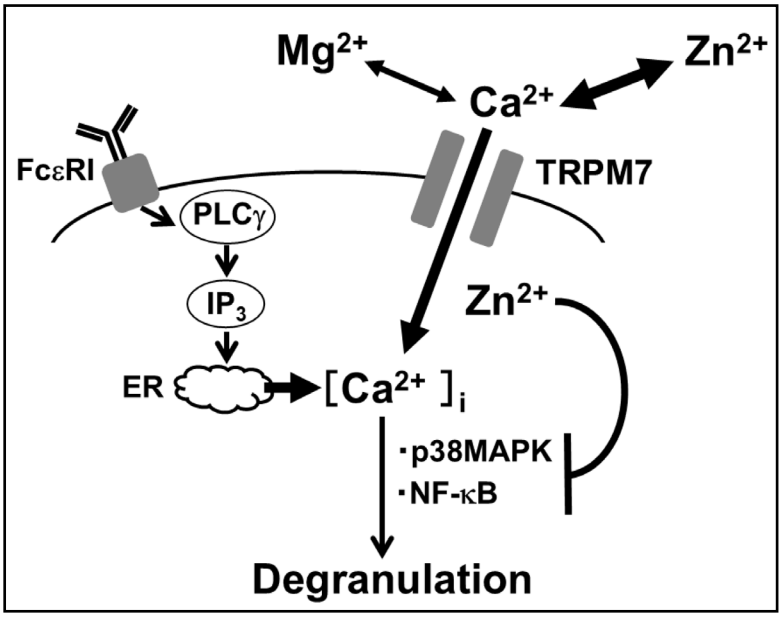

Fig. 5. Proposed mechanisms by which magnesium ions (Mg^{2+}) and zinc ions (Zn^{2+}) exert mast cell-stabilizing properties. Simulation of high-affinity IgE receptor (FceRI) results in the production of inositol triphosphate (IP_3) by phospholipase C-γ (PLC γ) and release of calcium ions (Ca^{2+}) through IP_3 receptors from Ca^{2+} stores in the endoplasmic reticulum (ER). Such-induced rise in the intracellular Ca^{2+} concentration mediates a signal for mast cell degranulation. Transient receptor potential cation channel subfamily M member 7 (TRPM7), which allows Ca^{2+} entry into cells, contributes to the rise in intracellular Ca^{2+} concentration in mast cells. As a divalent cation channel, since TRPM7 is also permeable to Mg^{2+} and Zn^{2+}, these cations interfere with Ca^{2+} entry into mast cells, causing the suppression of exocytosis. Because TRPM7 is much more permeable to Zn^{2+} than Mg^{2+}, Zn^{2+} interferes with the Ca^{2+} entry more strongly than Mg^{2+}. Additionally, once entering the cells, zinc modulates p38 mitogen-activated protein kinase (p38MAPK) or nuclear factor-kappa B (NF-κB) signaling pathways necessary for mast cell degranulation.

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Author contributions

IK and HS performed the experiments and analyzed the data. IK designed the experiments, interpreted the results, and wrote the manuscript. All the authors have read and approved the final version of the manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Miyagi University, which included ethical considerations.

Disclosure Statement

The authors have no competing interests to declare.

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