



Antipruritic effect of ursolic acid through MRGPRX2/MrgprB2-dependent inhibition of mast cell degranulation and reduced TSLP production

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ABSTRACT

Ursolic acid (UA), a pentacyclic triterpene, exhibits diverse pharmacological effects, including potential treatment for allergic diseases. It downregulates thymic stromal lymphopoietin (TSLP) and disrupts mast cell signaling pathways. However, the exact molecular mechanism by which UA interferes with mast cell action remains unclear. Therefore, the current study aimed to uncover molecular entities underlying the effect of UA on mast cells and its potential antipruritic effect, specifically investigating its modulation of key molecules such as TRPV4, PAR2, and MRGPRX2, which are involved in TSLP regulation and sensation.

Calcium imaging experiments revealed that UA pretreatment significantly suppressed MRGPRX2 activation (and its mouse orthologue MrgprB2), a G protein-coupled receptor predominantly expressed in mast cells. Molecular docking predictions suggested potential interactions between UA and MRGPRX2/MrgprB2. UA pretreatment also reduced mast cell degranulation through MRGPRX2 and MrgprB2-dependent mechanisms. In a dry skin mouse model, UA administration decreased tryptase and TSLP production in the skin, and diminished TSLP response in the sensory neurons. While PAR2 and TRPV4 activation enhances TSLP production, UA did not inhibit their activity. Notably, UA attenuated compound 48/80-induced scratching behaviors in mice and suppressed spontaneous scratching in a dry skin model.

The present study confirms the effective inhibition of UA on MRGPRX2/MrgprB2, leading to reduced mast cell degranulation and suppressed scratching behaviors. These findings highlight the potential of UA as an antipruritic agent for managing various allergy- or itch-related conditions.

1. Introduction

Ursolic acid (UA) is a pentacyclic triterpenoid compound found abundantly in various plants, including apples, cranberries, and elderflowers (Nguyen et al., 2021; Wozniak et al., 2015). Its structure includes a carboxylic group at C28, a β -hydroxy function at C3, and an alkene at C12–C13. These features contribute to its diverse biological activities including anti-inflammatory, anticancer, hepatoprotective, neuroprotective, antibacterial, and antiviral effects (Luan et al., 2022; Mlala et al., 2019).

UA is also believed to be beneficial for various allergic and itch conditions. It inhibits Fc ϵ RI-mediated mast cell degranulation, suppressing allergic mediators (Dhakal et al., 2021). Moreover, UA down-regulates IL-5, IL-13, and IL-17 in allergic asthma models (Kim et al., 2013). UA also alleviates mucus hypersecretion and collagen

deposition in allergic rhinitis (Sun et al., 2020, 2021). Additionally, it ameliorates atopic dermatitis symptoms by regulating TLR4/NF- κ B and Nrf2/HO-1 pathways (Wang et al., 2023). These findings suggest that UA may exert antipruritic effects by modulating mast cell activity and itch-related molecules.

Allergies are hypersensitivity responses involving the release of inflammatory mediators such as histamine and tryptase from mast cells. Typically, immunoglobulin E (IgE) binds to Fc ϵ RI on mast cells, initiating degranulation. Recent studies have identified multiple pathways of mast cell degranulation independent of IgE, particularly involving MRGPRX2 (Mas-related G protein-coupled receptor) and its mouse counterpart MrgprB2. These receptors play crucial roles in IgE-independent processes, releasing inflammatory mediators such as histamine, tryptase, and cytokines (McNeil et al., 2015; Roy et al., 2021). Activation of MRGPRX2/MrgprB2 triggers events that elevate

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intracellular calcium levels via coupling to $G\alpha_q$ (Cao et al., 2021).

Compound 48/80 (C48/80), a polymeric mast cell secretagogue, can induce mast cell degranulation by activating MRGPRX2/MrgprB2 without IgE involvement (Meixiong et al., 2019). This IgE-independent degranulation can be triggered by various substances (Dziadowiec et al., 2024). Activation of MRGPRX2 induces mast cell degranulation, releasing pro-inflammatory mediators and contributing to pruritus.

Identifying MRGPRX2/MrgprB2 as key receptors in IgE-independent mast cell degranulation underscores the need to study both IgE-dependent and IgE-independent pathways to understand mast cell biology and pruritus. Therefore, to explore the antipruritic properties of UA, it is crucial to investigate its impact on MRGPRX2/MrgprB2. Although UA inhibits IgE-dependent mast cell degranulation (Dhakal et al., 2021), its effects on MRGPRX2/MrgprB2-mediated IgE-independent degranulation and pruritus modulation remain unexplored.

Meanwhile, UA also downregulates thymic stromal lymphopoietin (TSLP), a cytokine that induces pruritus, by inhibiting the signaling cascade in mast cells (Moon et al., 2019). TSLP is produced by keratinocytes, possibly through PAR2 (protease-activated receptor 2) and TRPV4 (transient receptor potential vanilloid 4) (Lee and Shim, 2021). PAR2 is another GPCR expressed in keratinocytes, activated by serine proteases such as trypsin, tryptase, and kallikreins. Activation of PAR2 also results in increased intracellular calcium levels and TSLP production from keratinocytes (Kouzaki et al., 2009). Similarly, TRPV4 is an ion channel expressed in keratinocytes, and its activation leads to a rise in intracellular calcium, which triggers the release of TSLP and other pro-inflammatory mediators from keratinocytes (Luo et al., 2018). However, the precise molecular mechanisms through which UA suppresses TSLP production are unclear.

Therefore, the present study aimed to clarify whether UA has antipruritic effects. We hypothesized that UA might modulate MRGPRX2/MrgprB2 activities and impact TSLP production. The primary objective was to determine if UA can inhibit itch pathways by interacting with these itch-related receptors and molecules.

2. Materials and methods

2.1. Reagents

Ursolic acid (catalog #HY-N0140) was purchased from MedChemExpress (Monmouth Junction, NJ 08852, USA). Compound 48/80, GSK1016709A and SLIGRL-NH₂ were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2. Animals

All animal testing protocols were approved by Gachon University Institutional Animal Care and Use committee (GU1-2023-IA0026) and were performed in accordance with the Gachon University Institutional Animal Care and Use guidelines. Nine-week-old C57BL/6 male mice were randomly assigned to either the control or treatment group. The mice were maintained under a 12:12 h light:dark cycle and provided *ad libitum* access to food and water. The scratching behavior of mice was recorded and the number of scratching bouts and time spent for scratching were evaluated using the Solomon Coder program (<https://solomon.andraspeter.com>). One bout of scratching was defined as the movement of hind limbs near the nape until the mouse touches the floor.

2.3. Acetone/diethyl ether/water (AEW) dry skin mouse model

After shaving the back of the neck, mice were randomly assigned to either an AEW treatment or control group. The necks of the AEW treatment group were treated with acetone and diethyl ether (1:1) for 15 s, followed by washing with water for 60 s. Treatment was repeated twice daily (10:00 and 17:00) for 5 consecutive days as described previously (Lee and Shim, 2021). The control group mice were treated with

water instead of acetone/diethyl ether.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The levels of tryptase and TSLP were measured using mouse tryptase ELISA kit (catalog #RD-TPS-Mu; Reddot Biotech Inc., Kelowna, BC, Canada) and mouse TSLP ELISA kit (catalog #MTLP00; R&D Systems, Minneapolis, MN, USA), respectively, following the manufacturer's protocol.

2.5. Cell culture and cDNA transfection

HEK293T cell line was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Thermo Fisher, NY, USA) containing 10% fetal bovine serum (FBS, Gibco Thermo Fisher, NY, USA), 1% penicillin (Gibco Thermo Fisher, NY, USA) and 1% ZellShield® (Minerva Biolabs, Berlin, Germany). The cells were maintained at 37 °C in an incubator with a 5% CO₂ humidified atmosphere. For transfection, cells were transfected by FuGENE® HD transfection kit (Promega, Madison, WI, USA) with cDNAs of TRPV4 (NM_001177428), PAR2 (NM_005242), MRGPRX2 (NM_054030) and MrgprB2 (NM_175531). Manufacturer's protocol was followed, and further experiments were performed 24 h after the transfection.

2.6. Primary culture of mouse dorsal root ganglia (DRG)

Mouse dorsal root ganglia (DRG) were extracted as previously described (Adhikari et al., 2024). Briefly, mouse DRG neurons were prepared and incubated with 1.2 mg/mL collagenase (Worthington Biochemical, NJ, USA) for 40 min at 37 °C, followed by another 40 min incubation at 37 °C with 2.5 mg/mL trypsin (Gibco, Life Technologies). After incubation, cells were centrifuged at 30 g for 10 min and resuspended in neurobasal medium (Gibco) containing 10% FBS, 50–100 ng/mL nerve growth factor (Invitrogen, Gaithersburg, MD, USA), and 100 U/mL ZellShield®. The cells were then seeded in an 8-well Lab-Tek chambers treated with poly L-lysine (Thermo Fisher Scientific, Waltham, MA, USA) and incubated for 48 h in the presence of 95% humidity and 5% CO₂ at 37 °C. After 48 h, the calcium imaging experiment was conducted out.

2.7. Calcium imaging

Calcium imaging was performed following the procedure described previously (In Kim et al., 2023). Briefly, the intracellular calcium levels were determined using a fluorescence microscope (ECLIPSE Ti-U; Nikon, Tokyo, Japan; and Leica DMI8 inverted microscope; Leica Microsystems Ltd., Wetzlar, Germany) with the calcium-specific fluorescent dye, Fluo-3/AM (5 μM; Invitrogen, Carlsbad, CA, USA). Fluo-3/AM was mixed with 0.1% F127 in normal buffer solution (NBS: 140 mM NaCl, 5 mM KCl, 2 mM CaCl₂/EDTA, 0.5 mM MgCl₂, 10 mM glucose, and 5.5 mM HEPES, adjusted to pH 7.4), and loaded into the cells by incubating for 60 min at 37 °C. After 60 min of incubation, the cells were washed twice with NBS and replaced with fresh NBS. Following treatment with the compounds, the alterations in the fluorescent images were recorded on a computer connected to the microscope. The excitation and emission wavelengths were 488 nm and 515 nm, respectively. The intracellular calcium levels were expressed as the F/F₀ ratio, where F indicates the fluorescence intensity of the region of interest at a certain time point, and F₀ indicates the initial fluorescence intensity at 0 s. Image analysis was performed using the ImageJ software (NIH), with custom-made scripts for semi-automatic cell counting, calculation of the F/F₀ ratio, and image production.

2.8. Molecular docking

The three-dimensional (3D) structure of human MRGPRX2 (PDB ID:

7VV6) was retrieved from the Protein Data Bank (PDB) (<https://www.rcsb.org>) (Burley et al., 2023). The structure of murine MrgprB2 (UniProt ID: Q3KNA1) was obtained from the AlphaFold Protein Structure Database (Varadi et al., 2024). The two-dimensional (2D) structure of C48/80 was obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov>), while its 3D conformation was generated and optimized using Avogadro software (<http://avogadro.cc>, version 1.2.0) (Hanwell et al., 2012). The 3D structure of UA was also obtained from PubChem. Prior to docking simulations, the residue was removed, and hydrogen and gasteiger charges were added to the PDB models of MRGPRX2 and MrgprB2 using DockPrep. Similarly, hydrogen atoms and gasteiger charges were added to UA and C48/80 to prepare them as ligands for docking studies. AutoDock Vina (Eberhardt et al., 2021) was employed to perform the docking simulations, wherein UA and C48/80 were docked into the putative ligand-binding pockets of MRGPRX2 and MrgprB2. The best binding poses were determined based on the predicted binding affinity

scores. The resulting binding conformations were analyzed and visualized using UCSF Chimera software (version 1.12) (Pettersen et al., 2004).

2.9. Statistical analysis

All statistical analyses were performed using GraphPad Prism software (version 9). The data are presented as mean $\pm$ standard error. Unpaired t-tests were used for comparison between two groups and one-way analysis of variance (ANOVA) with Dunnett's multiple comparison post-test was utilized for groups of more than three. Fisher's exact test was used for comparing the responsiveness of the DRG neurons. A *p*-value of less than 0.05 was considered statistically significant.

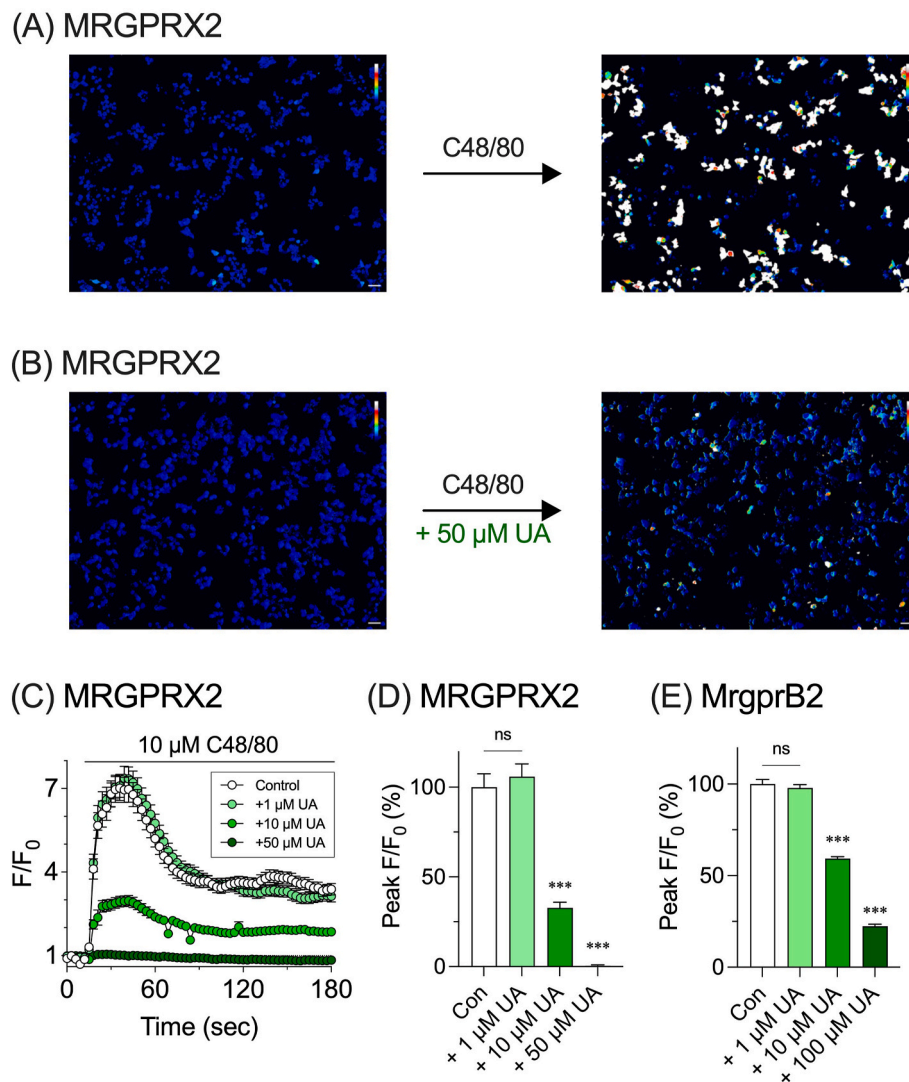


Fig. 1. UA inhibits the increase of intracellular calcium levels through MRGPRX2/MrgprB2. (A) Representative images of calcium imaging obtained from HEK293T cells transiently expressing human MRGPRX2. Although cells did not show changes in fluorescence levels in the medium (left), treatment with 10 μM compound 48/80 (C48/80) significantly evoked strong calcium-dependent fluorescence (right). (B) Similar to (A), but with pretreatment with 50 μM UA for 5 min before C48/80 application. Noticeably, most of cells showed no responses to C48/80 treatment. (C) Time-course profiles of calcium imaging experiments in HEK293T cells expressing MRGPRX2, with or without UA pretreatment. It was found that 10 μM and 50 μM UA showed inhibition, not 1 μM. (D) Comparison of the peak intensity of the calcium imaging experiments. Control (con) indicates no UA pretreatment (*n* = 393). The results showed that 1 μM UA was not sufficient for inhibition (*n* = 484), while 10 μM (*n* = 573) and 50 μM UA (*n* = 1315) showed a significant reduction. (E) Comparison of the peak intensity of calcium imaging experiments in cells expressing MrgprB2, a mouse orthologue of MRGPRX2. Control (con, *n* = 431) and 1 μM UA pretreatment (*n* = 869) did not show any difference, while pretreatment with 10 μM (*n* = 677) and 100 μM UA (*n* = 595) showed a significant decrease. *F* indicates the fluorescence intensity of the region of interest at a certain time point, and *F*₀ indicates the initial fluorescence intensity at 0 s. ns: not significant, ****p* < 0.001.

3. Results

3.1. UA inhibits MRGPRX2 and MrgprB2

We first investigated whether UA can affect the activity of MRGPRX2, a GPCR that is specifically expressed in mast cells. To this end, we transiently expressed MRGPRX2 cDNA in HEK293T cells and its activity was verified using the calcium imaging technique. Since MRGPRX2 is a GPCR linked to $G\alpha_q$, a subunit that leads to an increase in intracellular calcium levels when activated, its activation by the specific agonist compound 48/80 (C48/80) will induce an increase in calcium levels when MRGPRX2 is expressed.

As shown in Fig. 1A, our calcium imaging experiments revealed a strong increase in calcium-specific fluorescence when 10 μ M C48/80 was applied to cells expressing MRGPRX2. The C48/80-induced responses were concentration-dependent, and only observed in cells expressing MRGPRX2 (Supplementary Fig. 1). However, pretreatment with 50 μ M UA prior to C48/80 application resulted in a strong reduction in fluorescence intensity (Fig. 1B), suggesting that UA may inhibit C48/80-induced MRGPRX2 activation. While 50 μ M and 10 μ M of UA were shown to inhibit MRGPRX2 activity, 1 μ M of UA did not inhibit the increase in calcium levels (Fig. 1C and D). Similarly, for MrgprB2, a mouse orthologue of MRGPRX2, a comparable inhibitory pattern was observed, with significant inhibition observed at 10 μ M and 100 μ M UA (Fig. 1E).

Overall, it was found that UA has the ability to inhibit the activity of MRGPRX2 or MrgprB2 expressed in HEK293T cells, implying that UA may block some MRGPRX2/MrgprB2-dependent reactions in mast cells.

3.2. UA interacts with the binding pockets of MRGPRX2 and MrgprB2

Next, we investigated the potential interactions between UA and human MRGPRX2 through *in silico* molecular docking analysis. The results showed that C48/80 binds to amino acid residues in human MRGPRX2, located near the membrane surface. (Fig. 2A). Conversely, it was speculated that UA also binds to the same binding pocket but does not appear to be located in the exact same spot compared to C48/80

(Fig. 2B), indicating that UA might disrupt the binding of C48/80 to MRGPRX2.

Molecular docking of mouse MrgprB2 with UA was also conducted. As shown in Fig. 2C, C48/80 is predicted to interact with specific residues of MrgprB2. Similarly to MRGPRX2, it is speculated that UA also binds to the same binding pocket but does not appear to position itself exactly as C48/80 does (Fig. 2D), implying an inhibitory effect of UA over C48/80 in MrgprB2. Overall, these results demonstrate that UA may structurally interfere with the action of C48/80 in MRGPRX2/MrgprB2, potentially explaining why UA behaves as an antagonist for MRGPRX2/MrgprB2.

3.3. UA inhibits mast cell degranulation via inhibition of MRGPRX2/MrgprB2

Although it is well known that activation of MRGPRX2/MrgprB2 by C48/80 in mast cells results in degranulation (Meixiong et al., 2019), it is yet unclear whether UA can affect C48/80-induced mast cell degranulation or not. To verify this possibility, the level of β -hexosaminidase, an enzyme released during mast cell degranulation (Fig. 3A), was measured to verify whether the mast cell degranulation is affected by UA pretreatment. In HMC 1.2, a cell line originating from human mast cell, it was observed that C48/80-induced β -hexosaminidase levels were observed to be significantly decreased by 10 μ M UA (Fig. 3B), suggesting that UA can inhibit mast cell degranulation in HMC 1.2 cells. To further confirm the effect of UA on MrgprB2, mouse peritoneal mast cells (PMCs) were prepared. As shown in Fig. 3C, β -hexosaminidase assay showed that UA also inhibits mast cell degranulation of mouse PMCs (Fig. 3C), probably by inhibition of MrgprB2 activity. Therefore, these results imply that UA has the ability to inhibit mast cell degranulation via an MRGPRX2/MrgprB2-dependent manner.

3.4. UA downregulates TSLP production in the skin and decreases TSLP-induced intracellular calcium levels in the sensory neurons

Generally, TSLP is believed to be primarily produced in keratinocytes, with this production being induced by tryptase secreted from mast

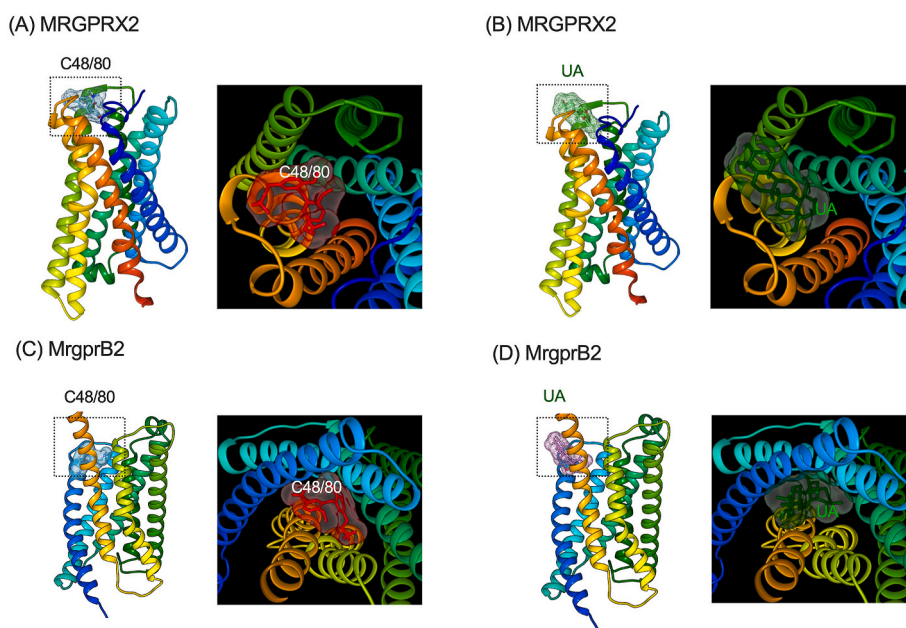


Fig. 2. UA is predicted to interact with the binding pockets of MRGPRX2 and MrgprB2. (A) Three-dimensional structure showing the putative interaction of Compound 48/80 (C48/80) with MRGPRX2. The square indicated by the dotted line indicates the region of the binding pocket, which is magnified on the right. (B) UA is also predicted to interact with key residues in MRGPRX2, although not all key residues are identical. (C) Similar molecular docking prediction was performed with mouse MrgprB2. Note that the binding pocket of C48/80 with MrgprB2 is similar to that of MRGPRX2. (D) UA also occupies the same binding pocket of C48/80 in MrgprB2.

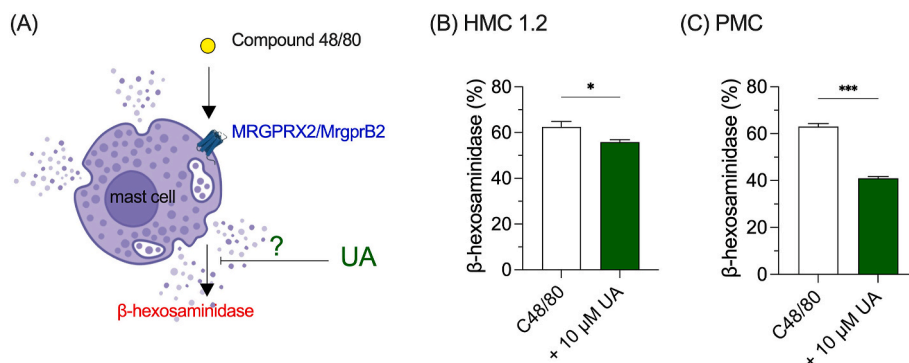


Fig. 3. UA inhibits mast cell degranulation. (A) A schematic illustration of β -hexosaminidase assay. Compound 48/80 acts on human MRGPRX2 and mouse MrgprB2, which evokes mast cell degranulation. During degranulation, various mediators such as histamine, tryptase, and β -hexosaminidase are released. Therefore, the level of β -hexosaminidase is proportional to the degree of mast cell degranulation. The aim was to investigate whether UA will affect on the mast cell degranulation by β -hexosaminidase assay. (B) HMC 1.2, a human mast cell line, showed decreased mast cell degranulation by 10 μ M UA treatment ($n = 3$). (C) Peritoneal mast cell (PMC) obtained from mice also showed decreased mast cell degranulation by 10 μ M UA treatment ($n = 3$). * $p < 0.05$, *** $p < 0.01$.

cells. Given that UA can decrease the level of TSLP in keratinocytes (Moon et al., 2019), it was pursued whether UA could indeed suppress TSLP production. To maximize the TSLP production in keratinocytes, AEW-treated mice, an animal model that develops dry skin condition and mast cell proliferation (Miyamoto et al., 2002), were prepared. While one group of mice were treated with AEW, another group of mice were treated with AEW along with UA (50 mg/kg), which was administered intraperitoneally, as illustrated in Fig. 4A.

After 5 days, the skin of the two groups were collected and levels of tryptase and TSLP were determined by ELISA. As a result, it was found that levels of both tryptase (Fig. 4B) and TSLP (Fig. 4C) in the mouse skin were significantly reduced in the skin of UA-treated AEW mice, confirming the ability of UA to inhibit TSLP production in the skin.

Once TSLP is produced from the keratinocytes, it is known that TSLP can act as a pruritogens by directly activating its cognate receptor expressed in the sensory neurons to increase the intracellular calcium levels (Wilson et al., 2013). Therefore, we investigated whether UA could alter the effect of TSLP on sensory neurons. As shown in Fig. 4D, pretreatment with 10 μ M UA significantly decreased intracellular calcium levels induced by 5 ng/mL TSLP. Furthermore, UA pretreatment also decreased the number of cells that are responsive to TSLP (Fig. 4E).

Overall, UA was found to not only inhibit TSLP production in the skin, but also decrease TSLP-induced responses in the sensory neurons in mice.

3.5. UA does not interact with TRPV4 and PAR2

Previous studies found that tryptase stimulates PAR2 (protease-activated receptor 2), which subsequently can activate TRPV4 to induce TSLP production in keratinocytes (Kouzaki et al., 2009; Lee and Shim, 2021). Since UA was found to inhibit TSLP production, it was investigated whether UA might inhibit PAR2 and/or TRPV4 to achieve its effect on TSLP (Fig. 5A).

Since PAR2 is a GPCR linked to $G\alpha_q$, its activation results in an increase in intracellular calcium levels. To investigate this, we prepared HEK293T cells expressing PAR2 and conducted calcium imaging experiments. However, as shown in Fig. 5B, UA did not significantly alter the time-course profiles of PAR2 activation by its agonist, SLIGRL-NH₂. Furthermore, we applied a similar approach to TRPV4 by transiently transfecting HEK293T cells with TRPV4 cDNA and performing calcium imaging experiments. As shown in Fig. 5C, pretreatment with 10 μ M UA did not affect the overall time-course profiles of TRPV4 activation by GSK1016709A, a TRPV4-specific agonist. This suggests that UA does not inhibit TRPV4.

In summary, UA did not interact with either TRPV4 or PAR2, indicating that its inhibitory effect on TSLP production is not associated with

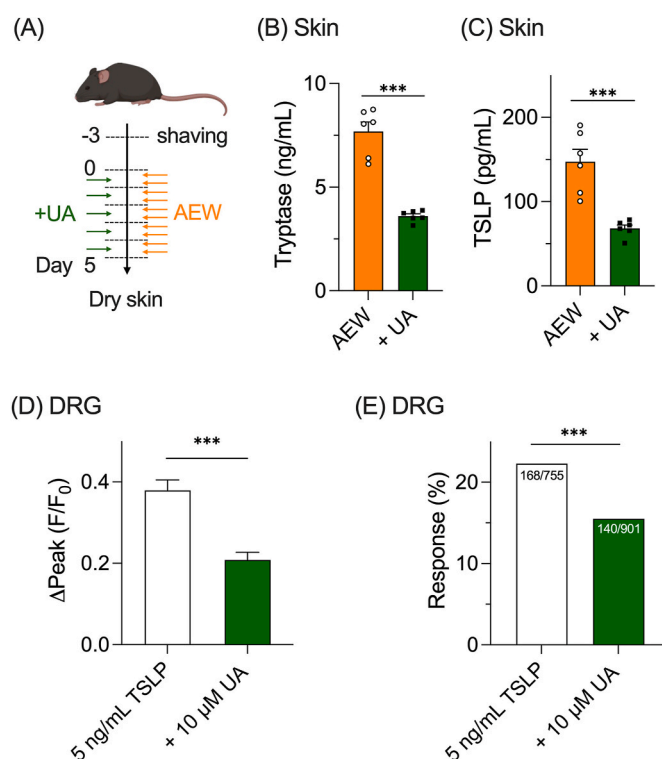


Fig. 4. UA reduces the production of TSLP and its impact on sensory neurons. (A) Schematic illustration of how the dry skin mice model is introduced by AEW (acetone, ethanol, and water). During AEW treatment for 5 days, 50 mg/kg UA was intraperitoneally administered daily. ELISA experiments showed that the levels of tryptase (B) and TSLP (C) in the skin of AEW mice treated with UA (" + UA", $n = 6$) were decreased in mice treated with control AEW ("AEW", $n = 6$). (D) Calcium imaging experiments with primarily-cultured dorsal root ganglia (DRG) sensory neurons showed that the peak F/F_0 induced by 5 ng/mL TSLP was significantly decreased by pretreatment with 10 μ M UA ($n = 901$) compared to control ($n = 755$). (E) Responses to 5 ng/mL TSLP in DRG neurons were compared between control and 10 μ M UA pretreated group. Fisher's exact test showed that UA pretreatment significantly reduced the response rate of DRG neurons to TSLP-induced calcium increase.

the inhibition of these molecules. Therefore, we speculate that the mechanism underlying the decreased TSLP production by UA is due to reduced production of tryptase, which in turn results in less signaling to produce TSLP in keratinocytes.

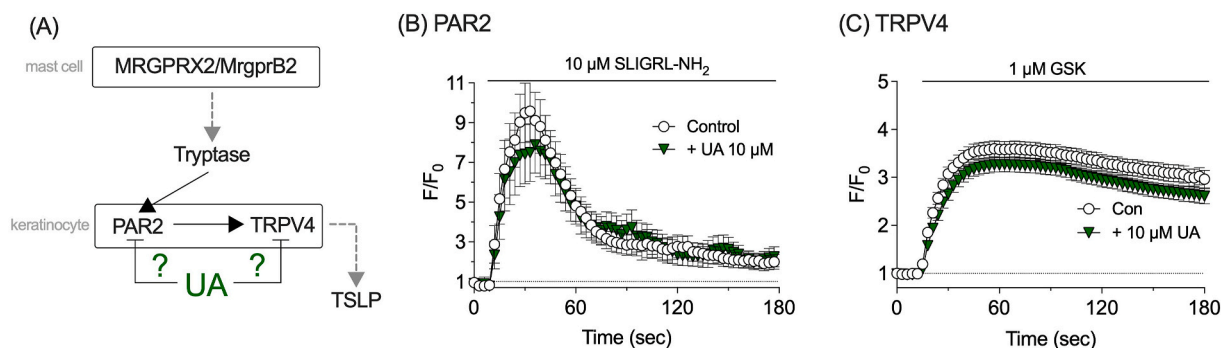


Fig. 5. UA does not inhibit the activities of TRPV4 and PAR2. (A) Activation of PAR2 and TRPV4 are known to induce TSLP production in keratinocytes (B) Pretreatment with 10 μ M UA also did not suppress PAR2 activation by 10 μ M SLIGRL-NH₂ ($n = 93$) compared to the control group ($n = 99$). (C) Pretreatment with 10 μ M UA did not inhibit the activation of TRPV4 by 1 μ M GSK1016709A ($n = 1396$) compared to control group ($n = 1238$).

3.6. UA can alleviate scratching behaviors in mice

Finally, to confirm the antipruritic effect of UA *in vivo*, scratching behavior was induced by administering C48/80 to the nape (300 μ g/site), with or without intraperitoneal injection of 50 mg/kg UA. As depicted in Fig. 6A and B, UA pretreatment significantly suppressed scratching behaviors induced by C48/80 (2.500 ± 1.310 bouts, $n = 6$) compared to the control groups (86.83 ± 16.77 bouts, $n = 6$). Furthermore, UA not only reduced the number of scratching bouts but also decreased the time spent scratching (2.167 ± 1.146 s, $n = 6$) when compared to the control (55.37 ± 9.676 s, $n = 6$), as shown in Fig. 6C. Additionally, as demonstrated in Fig. 6D, UA also suppressed spontaneous scratching behaviors in AEW mice (17.33 ± 6.458 bouts, $n = 6$)

compared to the control (65.17 ± 12.21 bouts, $n = 6$), suggesting that UA indeed exhibits an antipruritic effect in cases of TSLP-related dry skin conditions.

In conclusion, these findings confirm that UA possesses an antipruritic effect *in vivo*, underscoring its potential as a promising candidate for antipruritic therapy.

4. Discussion

Itch, initially perceived as a transient warning signal, can evolve into a severe, chronic condition that significantly diminishes quality of life. The management of itch remains challenging due to the complex molecular pathways involved in the peripheral nervous system. Current understanding divides itch-inducing signals in sensory neurons into two main categories: histamine-dependent and histamine-independent pathways (Sanjel and Shim, 2023). This classification depends on the involvement of histamine, an endogenous pruritogenic substance released by mast cells. Recent advances in understanding various histamine-independent pathways have highlighted the critical roles of numerous transient receptor potential (TRP) ion channels and G protein-coupled receptors (GPCRs) in itch signal transmission within sensory neurons. Consequently, contemporary itch research often focuses on investigating and modulating the activities of these TRP channels and GPCRs, which are crucial for itch signal transmission.

As mentioned earlier, UA exhibits a broad spectrum of beneficial pharmacological effects, with its anti-inflammatory properties being particularly noteworthy. Previous research has demonstrated the ability of UA to inhibit histamine release from rat mast cells, as shown in a study using UA extracted from *Melaleuca Leucadendron* (Tsuruga et al., 1991). Additionally, UA and oleanolic fraction derived from *Helichrysum plicatidii* have been reported to inhibit degranulation of rat peritoneal mast cells (Santos Rosa et al., 2007). The mechanism underlying the effects of UA was previously attributed to a membrane-stabilizing action that inhibited mast cell degranulation (Redei et al., 2018). However, the exact molecular targets remained unknown. Our study has now elucidated this mechanism, demonstrating for the first time that UA inhibits human MRGPRX2 and mouse MrgprB2, thereby suppressing mast cell degranulation. Interestingly, rat mast cells express MrgprB3, an orthologue of human MRGPRX2 and mouse MrgprB2, which mediates degranulation induced by compound 48/80 (Sahid et al., 2020). Given our findings, it is plausible that UA may also inhibit rat MrgprB3 activity through a similar mechanism, potentially explaining the previously observed effects in rat models.

Considering the diverse pharmacological effects of UA, it is likely that multiple mechanisms contribute to its antipruritic action. The antioxidant properties of UA, particularly its ability to protect against UVB-induced lipid peroxidation and oxidative DNA damage, are noteworthy in this context (Ramachandran and Prasad, 2008). Importantly, reactive oxygen species (ROS) play a significant role in the sensation of

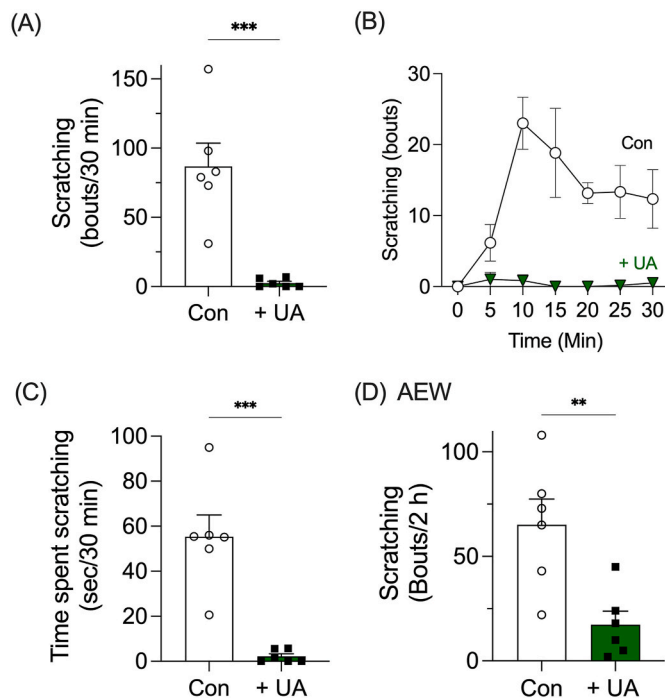


Fig. 6. UA reduces scratching behaviors in mice. (A) The total bouts of scratching induced by compound 48/80 (C48/80, 300 μ g/site) at the nape were significantly reduced ($n = 6$) when mice were administered intraperitoneally with 50 mg/kg of UA prior to injection of C48/80 ($n = 6$). (B) Time interval graph showing that scratching bouts were significantly suppressed throughout the period. (C) Total scratching time was also significantly reduced by UA pretreated compared to the control group injected with C48/80. (D) Spontaneous scratching bouts in AEW-treated control mice ($n = 6$) were significantly reduced by pretreatment with 50 mg/kg of UA ($n = 6$). ** $p < 0.01$, *** $p < 0.001$.

itch. For instance, TRPA1 channel is believed to play a crucial role in oxidant-induced pruritus (Liu and Ji, 2012). Moreover, a recent study revealed that mitochondrial ROS can trigger both acute and chronic itch in mice through activation of TRPC3 (Kim et al., 2022). Indeed, the involvement of ROS and resulting oxidative stress in pruritic conditions such as atopic dermatitis and psoriasis is well-documented (Biazus Soares et al., 2023). Given this connection, the antioxidant properties of UA may contribute significantly to its antipruritic effects. Our recent discovery of the antipruritic action of UA thus aligns with its overall beneficial impact and may be partially explained by its ability to mitigate oxidative stress.

In addition to our finding, UA is well known for its beneficial effect in skincare and itch management, as evidenced by a collection of studies. For instance, UA alleviates symptoms of atopic dermatitis model mice by regulation of pivotal signaling pathways such as TLR4/NF- κ B and Nrf2/HO-1 (Wang et al., 2023). Furthermore, topical formulations incorporating UA display promise in treating psoriasis, showcasing its versatility in managing diverse dermatological concerns (Miastkowska et al., 2023). A potential role of UA in enhancing skin condition is further highlighted by its ability to augment ceramides and collagen, essential for preserving skin barrier integrity and elasticity (Yarosh et al., 2000). Moreover, a study observed that UA can inhibit skin pigmentation by increasing melanosomal autophagy (Park et al., 2020), presenting UA as a comprehensive solution for maintaining skin tone. Specifically, UA suppressed increase of melanin contents in a manner dependent on α -MSH, which is known to elicit itch through the direct action of primary afferents in atopic dermatitis (Andoh et al., 2019). Altogether, these findings underscore the multifaceted benefits of UA, positioning it as a valuable component in skincare formulations targeting various dermatological issues, including itch relief and skin rejuvenation.

We found that the suppression of *in vivo* scratching behaviors by UA was relatively stronger than expected, especially compared to the *in vitro* calcium imaging results. While we speculate that the effect of UA is primarily due to the blockage of MRGPRX2/MrgprB2 and a decrease in TSLP production, other mechanisms may also contribute. For instance, the study by Dhakal et al. demonstrated that UA inhibits Fc ϵ RI-mediated mast cell activation and allergic inflammation by suppressing intracellular calcium levels and degranulation in a concentration-dependent manner (Dhakal et al., 2021). This suggests that UA may exert a potent antipruritic effect by inhibiting mast cell degranulation through both the IgE-dependent Fc ϵ RI-mediated pathway and the IgE-independent MRGPRX2-dependent mechanism. Therefore, the distinct inhibitory actions of UA on mast cell degranulation may explain its strong suppression of *in vivo* scratching behaviors compared to the *in vitro* calcium imaging results.

Although TSLP and C48/80 are not known to have a direct relationship, it is possible that TSLP sensitizes mast cells and sensory neurons, making C48/80 a more potent trigger for mast cell degranulation. In fact, a recent study found that pretreating mast cells with TSLP enhanced their degranulation (Babina et al., 2021). However, the administration of UA reduces TSLP production, thereby decreasing the likelihood of mast cell sensitization. Therefore, it is speculated that at least two mechanisms are involved in the antipruritic effect of UA observed in the present study: (a) an inhibitory effect of UA on MRGPRX2/MrgprB2, and (b) a reduction in TSLP production by UA, which prevents mast cell sensitization.

There are even other possible scenarios that may have contributed to the potent antipruritic effect of UA. For example, a report has found that UA can partially inhibit mast cell degranulation via lipid-centric plasma membrane perturbations (Shaik et al., 2022), implying that UA might have induced enhanced inhibitory effect on mast cell degranulation. In addition, while the present study shows that the antipruritic effect of UA occurs in the peripheral level, we cannot rule out the possibility that the antipruritic effect of UA is mediated in part by a central mechanism. For example, it is known that UA can exert anxiolytic-like or antidepressant-like effects (Colla et al., 2015), suggesting that UA can

affect its effects in the central nervous system as well. Indeed, UA is known to have therapeutic potential in common CNS disorders including traumatic brain injury, cerebral ischemia, cognition deficit, anxiety, and depression through its antioxidant and anti-inflammatory properties (Habtemariam, 2019). Further in-depth investigations are warranted to clarify the plausible reasons of strong antipruritic effect of UA.

While our findings clearly demonstrate the antipruritic effect of UA via MRGPRX2/MrgprB2 and TSLP production, our study has certain limitations. Primarily, we focused solely on MRGPRX2/MrgprB2-dependent TSLP-related itch pathways, potentially overlooking other interactions. Although MRGPRX2/MrgprB2-induced mast cell degranulation preferentially releases tryptase (Meixiong et al., 2019), mast cells also release histamine. Notably, UA appears to inhibit TRPV1 activity (Di et al., 2020; Verano et al., 2013), a key ion channel mediating histamine-induced itch signals through coupling with histamine receptor type 1 (Shim et al., 2007). Therefore, a comprehensive investigation is necessary to elucidate the role of UA in the histamine-induced itch pathway. In addition, it is still unclear how UA decreases the responsiveness of sensory neurons to TSLP. Future studies are warranted to clarify the uncertainties that remain unresolved in the current study.

In conclusion, the present study has demonstrated that UA efficiently inhibits MRGPRX2/MrgprB2, resulting in decreased mast cell degranulation. Additionally, UA reduces the production of TSLP and decreases scratching behaviors. These findings underscore the potential of UA as a therapeutic option for various allergy- or itch-related conditions.

CRediT authorship contribution statement

Jieun Cha: Writing – original draft, Investigation. **Juhee Ryu:** Writing – original draft, Investigation. **Diwas Rawal:** Software. **Wook-Joo Lee:** Validation, Investigation. **Won-Sik Shim:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors state no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejphar.2024.176896>.

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