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Citation	Jang, Yongwoo, Wook-Joo Lee, Gyu-Sang Hong, and Won-Sik Shim. 2015. "Red ginseng extract blocks histamine-dependent itch by inhibition of H1R/TRPV1 pathway in sensory neurons." Journal of Ginseng Research 39 (3): 257-264. doi:10.1016/j.jgr.2015.01.004. http://dx.doi.org/10.1016/j.jgr.2015.01.004.
Published Version	doi:10.1016/j.jgr.2015.01.004
Citable link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:17820899
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Research article

Red ginseng extract blocks histamine-dependent itch by inhibition of H1R/TRPV1 pathway in sensory neurons

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ABSTRACT

Background: Korean Red Ginseng—a steamed root of *Panax ginseng* Meyer—has long been used as a traditional medicine in Asian countries. Its antipruritic effect was recently found, but no molecular mechanisms were revealed. Thus, the current study focused on determining the underlying molecular mechanism of Korean Red Ginseng extract (RGE) against histamine-induced itch at the peripheral sensory neuronal level.
Methods: To examine the antipruritic effect of RGE, we performed *in vivo* scratching behavior test in mice, as well as *in vitro* calcium imaging and whole-cell patch clamp experiments to elucidate underlying molecular mechanisms.
Results: The results of our *in vivo* study confirmed that RGE indeed has an antipruritic effect on histamine-induced scratching in mice. In addition, RGE showed a significant inhibitory effect on histamine-induced responses in primary cultures of mouse dorsal root ganglia, suggesting that RGE has a direct inhibitory effect on sensory neuronal level. Results of further experiments showed that RGE inhibits histamine-induced responses on cells expressing both histamine receptor subtype 1 and TRPV1 ion channel, indicating that RGE blocks the histamine receptor type 1/TRPV1 pathway in sensory neurons, which is responsible for histamine-dependent itch sensation.
Conclusion: The current study found for the first time that RGE effectively blocks histamine-induced itch in peripheral sensory neurons. We believe that the current results will provide an insight on itch transmission and will be helpful in understanding how RGE exerts its antipruritic effects.
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1. Introduction

Itch is a sensation felt on the skin that causes a desire to scratch, a major symptom of many skin-related diseases. Although acute itch sensation is believed to play a role as an alerting system to eliminate pruritogens, chronic itch causes unwanted, debilitating, and uncontrollable scratching. This continuous scratching often induces inflammation and damages the skin barrier, resulting in an even more intensified itch sensation, and causing more severe scratching, a process known as a "vicious cycle."

Among many pruritogens, histamine has long been known as an endogenous mediator of itch sensation. The process is initiated by the activation of peripheral sensory neurons that project near to the skin. Activation of sensory neurons by a pruritogen produces an electrical signal in itch-mediating sensory neurons, which propagates into dorsal horns in the spinal cord; the signal then further leads up to the brain for final itch perception. In the case of a histamine-induced itch sensation, the histamine receptor subtype 1 (H1R) in the sensory neuron plays a significant role in transmitting the signal. Once histamine binds to H1R, the cascading pathway causes the activation of TRPV1 ion channel to produce an electrical signal in the itch-mediating sensory neurons [1]. In agreement with this finding, histamine-induced itch behavior was significantly reduced in TRPV1$^{-/-}$ mice [1], suggesting that

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histamine-dependent itch is mediated primarily through the actions of H1R and TRPV1. Thus, H1R and TRPV1 are considered to represent the histamine-dependent itch-signaling pathway.

Korean Red Ginseng—a steamed root of *Panax ginseng* Meyer—has long been used in Asian countries as a traditional medicine for treatment of various diseases [2]. In particular, the Korean Red Ginseng extract (RGE) has long been used for rejuvenation, and its usefulness has already been demonstrated in many experimental studies. For example, saponin and polyphenol components in red ginseng are crucial for the prominent protective effect on carcinogenesis and viral infection [3–5]. In various diabetic animal models, red ginseng administration showed a hypoglycemic effect by improving insulin secretion in pancreatic β cells [6–8].

Recently, it has been reported that red ginseng also has an antipruritic effect. Indeed, administration of red ginseng or saponin fraction inhibited scratching behavior in mouse models induced by either compound 48/80 or histamine [9]. In addition, in an experimental mouse model with atopic dermatitis, RGE reduced frequent scratching behavior and improved severe skin lesions [10,11]. In these studies, the decreased levels of immune-related mediators such as tumor necrosis factor-α, interferon-γ, and interleukin-4 were suggested as a possible mechanism of antipruritic effect by red ginseng [9,11]. However, considering that the itch sensation in the skin is exclusively transmitted via peripheral sensory neurons, chances are that red ginseng may also inhibit the itch sensation at the sensory neuronal level. To date, no studies investigating the antipruritic effect of red ginseng with this perspective have been reported.

Therefore, in the current study, we investigated whether RGE has any inhibitory effect on the histamine-dependent itch pathway in sensory neurons.

2. Materials and methods

2.1. Materials

Korean RGE was manufactured by Korea Ginseng Corporation (Seoul, Korea) from the roots of 6-year-old red ginseng, *P. ginseng* Meyer, harvested in the Republic of Korea. Briefly, Korean Red Ginseng was made by steaming fresh ginseng at 90–100°C for 3 hours and then drying at 50–80°C. The extract was prepared from red ginseng water extract, which was prepared by circulating hot water (85–90°C) three times. The water content of the pooled extract was 34.41% of total weight. High-performance liquid chromatography (HPLC) analysis showed that Korean RGE contained the following ginsenosides: Rb1, 7.53 mg/g; Rc, 2.98 mg/g; Rb2, 2.86 mg/g; Rg3, 2.09 mg/g; Re, 1.90 mg/g; Rg1, 1.78 mg/g; Rf, 1.12 mg/g; Rg2s, 1.12 mg/g; Rd, 0.89 mg/g; and Rh1, 0.84 mg/g.

2.2. Scratching behavior in vivo experiments

Mice of the Institute of Cancer Research (ICR) strain, 6–8 weeks old, were purchased from Orient Laboratory Animals (Seoul, Korea). To elicit scratching behavior, 100 μL of histamine (5 μg/site) was administered by subcutaneous injection into the neck. Immediately after histamine injection, bouts of scratching by hind limbs were counted for 1 hour, and video-recorded. To evaluate its antipruritic effect, Korean RGE was administered intraperitoneally 30 minutes prior to histamine injection.

2.3. Isolation of dorsal root ganglion neurons

For electrophysiological recordings, lumbar and thoracic dorsal root ganglion (DRG) neurons were isolated from adult mice (7–10 weeks), cultured as modified, as described previously [12]. The culture medium, Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1, Life Technologies, St. Louis, MO, USA), contained 10% fetal bovine serum, 100 U/mL penicillin/streptomycin, 50 ng/mL NGF (Invitrogen, Grand Island, NY, USA), and 5 ng/mL GDNF (Invitrogen, Grand Island, NY, USA). Isolated DRGs were collected in DMEM/F12 and incubated with 2 mg/mL Collagenase IA (Sigma-Aldrich) in DMEM/F12 for 45 minutes at 37°C. Cells were washed using HBSS (Life Technologies, St. Louis, MO, USA) and triturated, followed by plating on 20 mg/mL Laminin (Sigma-Aldrich)-coated glass coverslips. Electrophysiological recordings were performed on Day 2 within 24 hours.

2.4. Cell culture and gene transfection

HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum and 100 units/mL penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO_2. The cells were subcultured every 3–4 days with fresh DMEM medium for maintenance. Then, cells were transfected with appropriate genes (H1R and/or TRPV1 subcloned into pcDNA3.1 plasmid) using a FuGENE HD transfection reagent (Promega, Madison, WI, USA) according to the manufacturer's protocol. Each gene was transfected at a final concentration of 2 μg, and all experiments were performed 1 day after transfection. Genes used in this study—mouse H1R and mouse TRPV1—were previously cloned from mouse DRG, and matched 100% sequence identity with those in the National Center for Biotechnology Information Genbank database.

2.5. Electrophysiology

For DRG neuron recordings, the intracellular solution contained 130mM CsCl, 2mM $MgCl_2$, 5mM ethylene glycol tetraacetic acid (EGTA), 10mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 20mM D-mannitol, 2mM Mg-Adenosine triphosphate (ATP), and 0.2mM Na-Guanosine-5′-triphosphate (GTP), and the pH level was adjusted to 7.2 using CsOH. The extracellular solution contained 130mM NaCl, 5mM KCl, 1mM $CaCl_2$, 2mM $MgCl_2$, 10mM HEPES, and 10mM D-mannitol, and pH was adjusted to 7.2 using NaOH. Currents were sampled at 200 Hz and analyzed using pClamp software (version 6.0; Molecular Device, Sunnyvale, CA, USA).

2.6. Measurement of intracellular Ca^{2+}

Intracellular Ca^{2+} measurement was performed using a calcium imaging technique following a previously described method [13], using an inverted microscope (ECLIPSE Ti-U; Nikon, Tokyo, Japan). Briefly, HEK293T cells were cultured on a lysine-coated eight-well chamber (Lab-Tek; Thermo Scientific), and the grown cells were transfected with H1R and/or TRPV1 cDNA. One day after transfection, the culture media were replaced with normal bath solution [140mM NaCl, 5.0mM KCl, 2mM $CaCl_2$, 0.5mM $MgCl_2$, 10mM glucose, and 5.5mM HEPES (pH7.4)]. For intracellular calcium detection, calcium-specific fluorescent dye Fluo-3 AM (5μM, Invitrogen) was incubated for 1 hour in the presence of 0.1% Pluronic F-127 (Invitrogen). Following incubation, the medium was washed out, and appropriate compounds were applied to elicit calcium influx. The calcium-specific fluorescence was excited at 488 nm and emitted at 515 nm. Microscopic images were created using Nikon NIS Elements software at 1.5-second intervals. Changes in intracellular calcium levels are expressed as the F/F_0 ratio, where F_0 is the initial fluorescence intensity. Calculation of the ratio was performed using the ImageJ program (National Institutes of Health (NIH), Bethesda, MD, USA) with custom-made scripts.

2.7. Statistical analysis

All data were presented as mean ± standard error of the mean. One-way analysis of variance with Tukey's *post hoc* test was used for comparison of multiple means. A *p* value < 0.05 was considered significantly different. Statistical analysis was performed using Graphpad Prism 6 (Graphpad Software, La Jolla ,CA, USA).

3. Results

3.1. RGE inhibits histamine-induced scratching in vivo

To confirm the antipruritic effect of the Korean RGE, first we intraperitoneally preadministered RGE in mice. After 30 minutes, histamine was administered subcutaneously in the neck, and bouts of scratching were counted for up to 1 hour. As shown in Fig. 1B, severe scratching (88.00 ± 17.91 total bouts, *n* = 6) was evoked in mice treated with histamine alone (5 µg/site). However, bouts of scratching were significantly reduced (20.14 ± 5.064 total bouts, *n* = 7) in mice pretreated with RGE (50 mg/animal) prior to the application of histamine. As shown in Fig. 1A, pretreatment using 50 mg of RGE successfully reduced histamine-induced scratching behavior throughout all time ranges. In contrast, lower doses—e.g., 5 mg (85.75 ± 32.04 total bouts, *n* = 4) and 10 mg (65.67 ± 41.90 total bouts, *n* = 3)—of RGE failed to inhibit scratching behaviors, suggesting that the antipruritic effect of RGE has a dose-dependent tendency. Overall, it was confirmed that RGE has an antipruritic capability against histamine-induced scratching behaviors in mice.
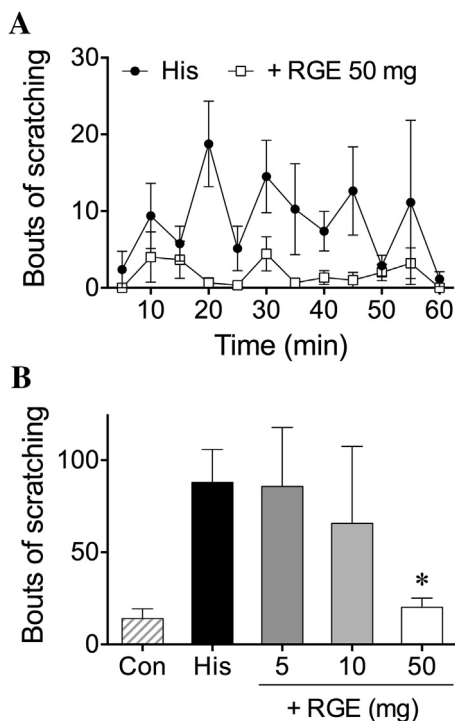

Fig. 1. Korean Red Ginseng extract (RGE) inhibits histamine-induced scratching in mice. RGE was intraperitoneally injected 30 minutes prior to histamine application, and 5 µg/site of histamine was administered subcutaneously into the dorsal neck of 6–8-week-old ICR mice. (A) A time-course graph for histamine-induced scratching between mice that did not receive pretreatment (His, filled circle, *n* = 6) versus mice pretreated with 50 mg/animal RGE (+RGE 50 mg, empty square, *n* = 7) for 1 hour. (B) Total bouts of histamine-induced scratching (5 µg/site) among different doses (5-, 10-, and 50-mg/animal) of RGE pretreatment. ANOVA with Tukey's *post hoc* test was applied for statistical analysis. Error bars indicate standard error mean. * *p* < 0.05. ANOVA, analysis of variance; ICR, Institute of Cancer Research strain.

3.2. RGE can inhibit histamine responses in mouse sensory neurons

To investigate the effect of RGE on sensory neurons, primary cultures of mouse DRG were tested to determine whether RGE has any inhibitory effects on histamine-induced actions. In whole-cell patch clamp experiments, application of histamine (100µM) alone evoked an inward current, as shown in Fig. 2A. However, when RGE (100 µg/mL) was applied after histamine was first treated, treatment with RGE resulted in a reduction of the inward current induced by histamine (Fig. 2B).As shown in Fig. 2C, the peak current by RGE cotreatment was significantly decreased [118.9 ± 19.49 pA (control, *n* = 7) vs. 29.98 ± 6.352 pA (+RGE, *n* = 7)]. Moreover, the recovery time has also been decreased by RGE cotreatment as shown in Fig. 2D [24.44 ± 6.929 minutes (control, *n* = 5) vs. 7.633 ± 2.892 minutes (+RGE, *n* = 5)]. Taken together, these data indicate that RGE has inhibitory effects against histamine-induced currents in sensory neurons.

Because histamine application induces calcium influx in DRG, the calcium imaging technique was applied for detection of histamine-induced responses. As shown in Fig. 2E and F, application of histamine (10µM) on mouse DRG evoked a strong increase in intracellular calcium. Surprisingly, this histamine-induced intracellular calcium increase was dramatically reduced by pretreatment with 100 µg/mL RGE (Fig. 2E and F). Of particular interest, the lower concentration of RGE (10 µg/mL) pretreatment was not sufficient to inhibit histamine-induced intracellular calcium increase. In addition, we have measured how many cells reacted to histamine application. Among 100 randomly selected cells, treatment of histamine alone evoked 12 cells to be responsive. However, pretreatment of RGE resulted in reduced numbers of histamine-responsive cells (9 for 10 mg/mL, 5 for 100 mg/mL), suggesting that RGE pretreatment has an inhibitory effect on histamine-induced responses on DRG neurons.

3.3. RGE can inhibit histamine-induced H1R/TRPV1 pathway

To further investigate the underlying molecular mechanisms of RGE on histamine-induced responses, we used two membrane proteins, H1R and TRPV1, because they are the main signal transducers in the histamine-induced itch pathway [1]. Thus, HEK293T cells transiently coexpressing H1R and TRPV1 (H1R/TRPV1) were prepared, and histamine was applied thereafter. Because TRPV1 is a nonselective cation channel that allows calcium influx when activated, the calcium imaging technique was applied as well. As shown in Fig. 3, treatment of 10µM histamine significantly induced intracellular Ca^{2+} increase in H1R/TRPV1 cells, indicating that the cell lines were functional and measurement of histamine-induced responses was accurate. With pretreatment of a low concentration of RGE (1 µg/mL and 10 µg/mL), the histamine-induced response did not differ from that of the control group, suggesting a lack of inhibition on histamine-induced activation of H1R/TRPV1. However, with pretreatment of a high concentration of RGE (100 µg/mL), the histamine-induced response was dramatically decreased (Fig. 3C).

These results demonstrate that RGE has an inhibitory effect on the H1R/TRPV1 pathway in a concentration-dependent manner.

3.4. RGE has mixed effects on capsaicin-induced activation of TRPV1

Finally, we attempted to examine the particular mechanism of the inhibition by RGE in the H1R/TRPV1 pathway. Although a couple of different signaling mechanisms between H1R and TRPV1 have been reported, TRPV1 activation is, without a doubt, the ultimate step in the H1R/TRPV1 pathway. In other words, it might be
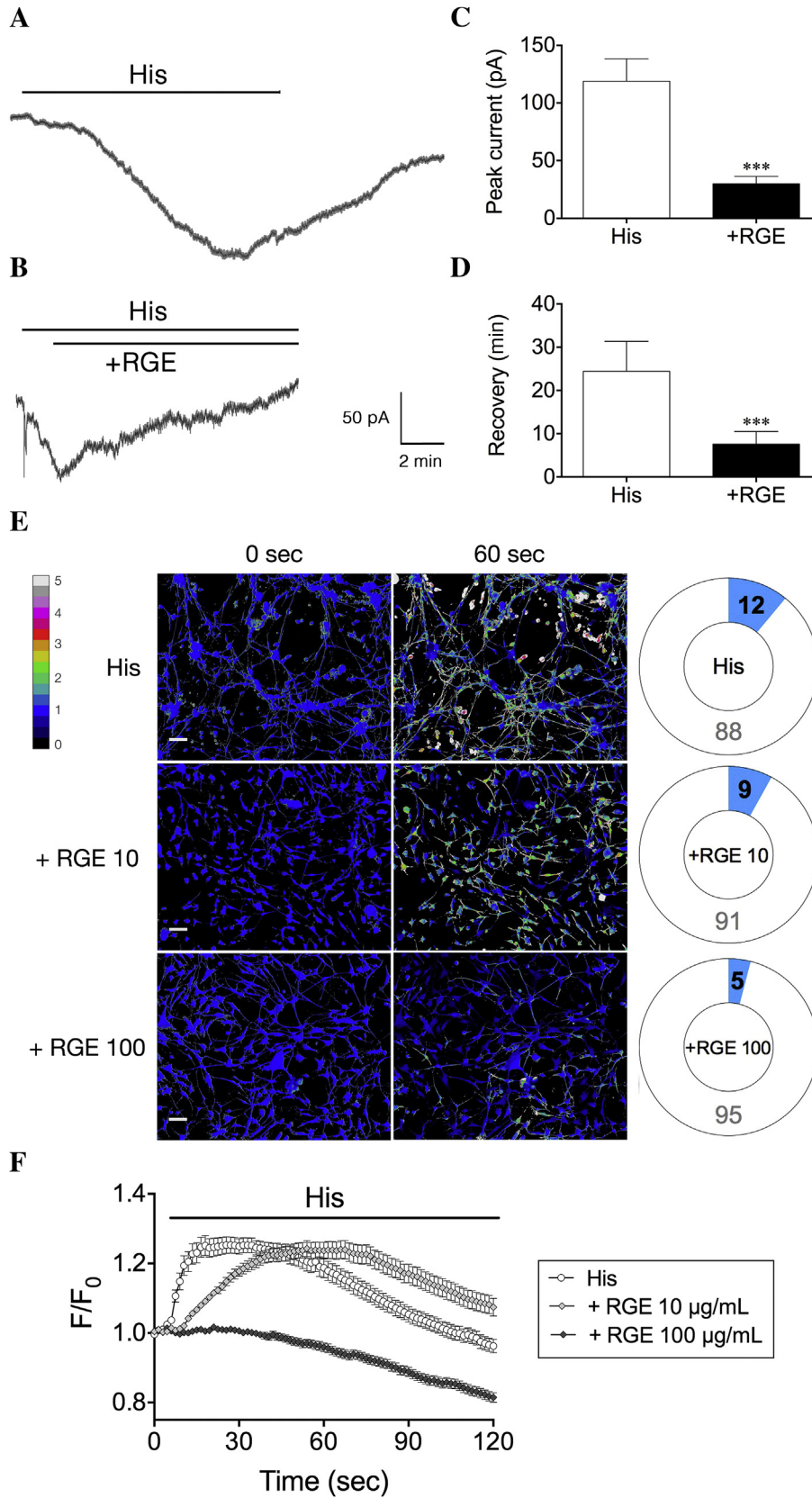
Fig. 2. RGE blocks histamine-induced responses in primary culture of mouse dorsal root ganglia (DRG). (A) A representative current induced by 100μM histamine (His) in mouse DRG with whole-cell configuration. (B) When RGE is added during histamine application, the current started to recover to the basal levels faster. (C) Summary of peak currents induced by 100μM histamine alone (His, white, $n = 7$) or RGE-added cells (+RGE, black, $n = 7$). *** $p < 0.001$ with Student t test. (D) Summary of recovery time between the two groups. Recovery time was defined as time that elapsed until currents reverted to 95% of the basal level. *** $p < 0.001$ with Student t test. (E) Representative ratiometric calcium

important to determine whether RGE has a *direct* inhibitory effect on TRPV1. Thus, the effect of RGE on TRPV1 alone was investigated.

As shown in Fig. 4, treatment with 1µM capsaicin—a TRPV1-specific agonist—resulted in significantly increased intracellular calcium levels, indicating that TRPV1 was functional without any problems. However, with pretreatment of 10 µg/mL of RGE, treatment with 1µM capsaicin not only failed to inhibit the responses, but—unexpectedly—the pretreatment rather potentiated the capsaicin responses (Fig. 4B), indicating that 10 µg/mL of RGE pretreatment apparently enhanced capsaicin-induced TRPV1 currents.

When a high concentration of RGE (100 µg/mL) was used, however, RGE pretreatment resulted in marginally decreased capsaicin-induced responses, although the inhibition was not dramatic as in histamine-induced experiments. In the current study, it was concluded that capsaicin-induced activation of TRPV1 was rather potentiated by a low concentration of RGE (10 µg/mL) but slightly inhibited by a high concentration of RGE (100 µg/mL). It is possible to assume that some ingredients in RGE might have opposing effects on TRPV1 activation, but it remains elusive at this point. Nevertheless, it can be summarized that there are mixed effects of RGE pretreatment over capsaicin-induced activation of TRPV1.

Overall, the current study showed that RGE has an inhibitory effect on histamine-induced itch sensation by inhibition of the H1R/TRPV1 pathway present in sensory neurons. It appears that RGE has a prominent antipruritic effect against histamine-induced itch at peripheral sensory neuronal levels. Although further investigation of the effect of RGE on TRPV1 itself is needed, the current study clearly demonstrated that RGE is indeed effective in alleviating itch-related symptoms, probably via inhibition of the histamine-induced H1R/TRPV1 pathway in sensory neurons.

4. Discussion

Inhibition of itch is important because it not only alleviates uncomfortable sensation, but also prevents further scratching, which often leads to aggravation of skin conditions. Although RGE has long been used in some Asian countries, the exact molecular mechanism of RGE on itch sensory neurons was rather obscure. The current study demonstrated for the first time that RGE has an inhibitory effect on the H1R/TRPV1 pathway in histamine-induced itch sensory neurons.

The effect of RGE on scratching or skin lesions, particularly in an atopic dermatitis animal model, had already been reported in the literature [9–11,14]. In agreement with these reports, the current study also found that RGE indeed has an antipruritic effect against histamine-induced scratching in ICR mice (Fig. 1). Of particular interest, the effect was only visible when a high dose (50 mg, Fig. 1B) of RGE was administered, not in low doses (5 mg or 10 mg, Fig. 1B). Given that RGE is a mixture of various compounds, including many ginsenosides, only 50 mg of RGE may contain sufficient amounts of ingredient(s) required for scratching inhibition. Considering that ginsenoside Rg3 have an antipruritic effect *in vivo* [9], the antipruritic effect of RGE might be largely attributable to Rg3. However, because there may be other ginsenosides with yet unknown antipruritic effects, further investigation is necessary for the identification of all single compound(s) responsible for the antipruritic effect in RGE.

It is generally accepted that itch stems from the activation of peripheral sensory nerve endings in response to various pruritogens [15]. Most pruritus-generating sensory neurons are unmyelinated C-fibers and myelinated Aδ-fibers [16,17], and the major group is TRPV1-positive sensory neurons [18]. TRPV1-positive sensory neurons are known to transduce the itch signal by responding to histamine, which binds to itch-specific H1R [19]. Thereafter, the signal cascade triggers the production of an endogenous TRPV1 activator, 12-hydroxyeicosatetraenoic acid, via phospholipase A_2 and 12-lipoxygenase pathway [1]. The signal induced by TRPV1 activation leads toward the secondary pruriceptor in the dorsal horn of the spinal cord, which transduces an itch signal to the brain [20]. Thus, if any compound can inhibit the H1R/TRPV1 pathway, it could have an inhibitory effect against histamine-induced itch.

However, it should also be mentioned that H1R is not the only receptor that relays the histamine-mediated itch. Indeed, among the four different subtypes of histamine receptors, H4R is also known to be a receptor responsible for histamine-induced itch along with H1R. Although H4R is mostly expressed on immune cells, it is considered to be a new therapeutical target for many itch-related diseases [21]. Therefore, we cannot exclude the possibility that RGE may act on H4R to induce an antipruritic effect; however, this topic is beyond the scope of the present study and needs further investigation.

Because most sensory neurons have their cell bodies in the DRG, the primary culture of DRG provides excellent experimental conditions for testing various effects of compounds on sensory neurons. In this context, the data shown in Fig. 2 are noteworthy, because they proved for the first time that RGE indeed has an inhibitory effect against histamine-induced responses in sensory neuronal levels. Similar to *in vivo* tests, it was found that only a high concentration of RGE (> 100 µg/mL) was required to exert the inhibitory effect, implying that the key single compound(s) in RGE might not be sufficient at a low concentration of RGE (10 µg/mL). This pattern of dose-dependent response was also found in HEK293T cells expressing H1R/TRPV1 (Fig. 3), strongly supporting our hypothesis. Therefore, it is possible to assume that the key ingredient(s) of RGE for inhibition of histamine-induced responses might be rather a *minor* component in terms of total amount, because only a high concentration of RGE turned out to be effective. However, this argument could be true when there are ingredients with opposing actions on TRPV1 as well. Thus, further thorough investigation is warranted to clarify this speculation.

This dose-dependent effect of RGE was also found in the blocking effect on capsaicin-induced TRPV1 activation (Fig. 4). For unknown reasons, a low concentration of RGE (10 µg/mL) not only failed to inhibit the capsaicin-induced responses, but it rather *potentiated* TRPV1 actions. Although this seemed odd at first, it was later found that the phenomenon was not completely new. In fact, it was in agreement with a previous report that certain ginsenosides in red ginseng, such as Rc, potentiate capsaicin-induced responses on TRPV1 expressed in *Xenopus* oocytes [22]. Thus, it can be assumed that some ginsenosides, such as Rc, might have potentiated the capsaicin-induced TRPV1 actions in our experiments as well. Considering that the RGE used in the current study also contains Rc (2.98 mg/g), it seems possible that the potentiation was attributable to Rc; however, further verification is required.

Interestingly, this TRPV1 potentiation phenomenon was less evident when a higher concentration (> 100 µg/mL) of RGE was used. One plausible explanation would be that a high concentration of RGE contains sufficient amounts of minor ginsenosides, which

imaging data showing the effect of RGE (10 µg/mL and 100 µg/mL) over histamine-induced responses. Donut-shaped diagrams indicate how many cells were responsive to the stimuli. Cells were regarded as responsive when F/F_0 is > 1.8. Notice that 100 µg/mL of RGE significantly blocked histamine-induced Ca^{2+} influx. Pseudocolors represent F/F_0, where F indicates the fluorescence intensity of calcium-specific dye and F_0 indicates the initial fluorescence intensity. Scale bar = 100 µm. (F) Time-course traces of calcium imaging with histamine with or without RGE pretreatment up to 2 minutes. RGE, red ginseng extract.
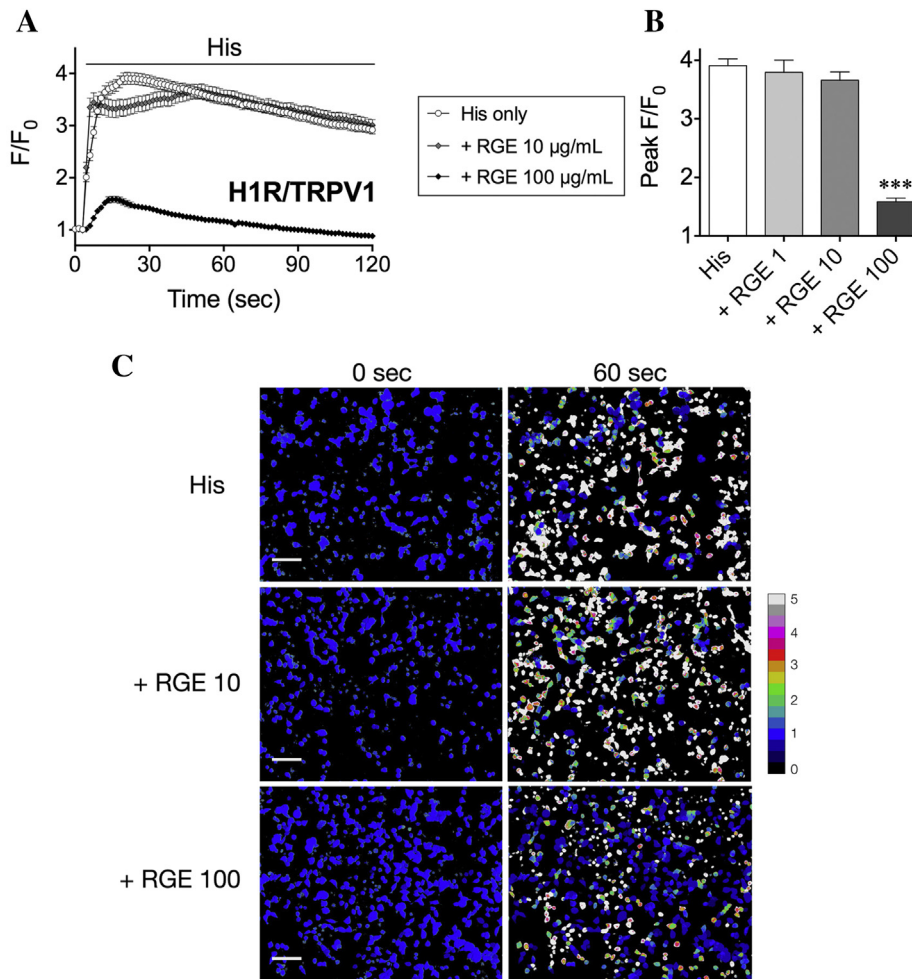
Fig. 3. RGE inhibits histamine-induced H1R/TRPV1 pathway. (A) Traces of Ca^{2+} influx induced by histamine in the absence (empty circle, $n = 1873$) or presence (10 μg/mL: gray, $n = 918$, 100 μg/mL: black, $n = 1123$) of RGE for up to 120 seconds in HEK293T cells transiently coexpressing H1R and TRPV1 (H1R/TRPV1). (B) Summary of peak Ca^{2+} influx (F/F_0) induced by either histamine alone or histamine pretreated with RGE. *** $p < 0.001$, ANOVA with Tukey's *post hoc* test. (C) Representative ratiometric calcium imaging data of histamine-induced Ca^{2+} influx before and after treatment of 10 μg/mL or 100 μg/mL RGE at 60 seconds. Scale bar = 100 μm. ANOVA, analysis of variance; H1R, histamine receptor subtype 1; RGE, red ginseng extract.

may exert inhibitory action on the capsaicin-induced action of TRPV1. In other words, RGE contains various ingredients, wherein one fraction *potentiates* capsaicin-induced responses, whereas the other rather *inhibits* the capsaicin-induced action of TRPV1. Based on our own results, it seems that the potentiating fraction is more abundant than the inhibitory fraction in RGE, because a low concentration of RGE (10 μg/mL) only potentiated the capsaicin-induced action of TRPV1. However, with pretreatment of a high concentration of RGE (100 μg/mL), the capsaicin-induced response was decreased, but not completely. This may be attributed to the result of a net TRPV1 response by two contradictory fractions of RGE. Again, the inhibitory effect of RGE only became evident under high concentration, which strongly suggests that the inhibitory fraction may be a minor component in RGE. The exact mechanism of how this potentiation/inhibition of TRPV1 occurs is not clear; thus, further investigation is absolutely necessary.

One may wonder why there is no potentiation effect of RGE-—especially for 10 μg/mL—on histamine-induced responses in H1R/TRPV1 cells. Although we do not have concrete reasons for this, it can be speculated that the activation mechanism of TRPV1 might differ by agonists. Unlike capsaicin-induced activation of TRPV1, histamine-induced activation is believed to produce 12-(S)-HPETE, an endogenous agonist for TRPV1 [23]. Thus, it is reasonable

to presume that the activation mechanisms of TRPV1 by capsaicin and 12-HPETE could be different, thereby causing dissimilar effects of RGE on TRPV1 in the end. Indeed, it was found that activation of TRPV1 by 12-(S)-HPETE contrasts with the rapid activation by capsaicin [23], implying that at least the activation kinetic of TRPV1 is different by these agonists. Nonetheless, it was clear that at least RGE does not have any potentiation effect on the histamine-induced H1R/TRPV1 pathway. As shown in Fig. 1, our *in vivo* results already demonstrated that histamine-induced scratching was not exacerbated by a low dose of RGE. This could be important because it may indicate that RGE will not cause enhanced itch sensation after all.

Because TRPV1 is an ion channel that is mostly involved in pain sensation, it may sound odd that TRPV1 is also involved in itch, which is a distinctly different class of sensation. However, it is now believed that interpretation of sensation may be "fiber-dependent" even though TRPV1 is found in both itch and pain peripheral sensory neurons: When TRPV1 in pain sensory neuron is activated, it is of course interpreted as pain. However, activation of TRPV1 in itch fiber will specifically produce an itch sensation, probably because itch-specific sensory neurons have their own neurotransmitter, such as GRP (gastrin-related peptide), and itch its counterpart receptor (GRPR) in the spinal cord [24]. The notion of this "fiber
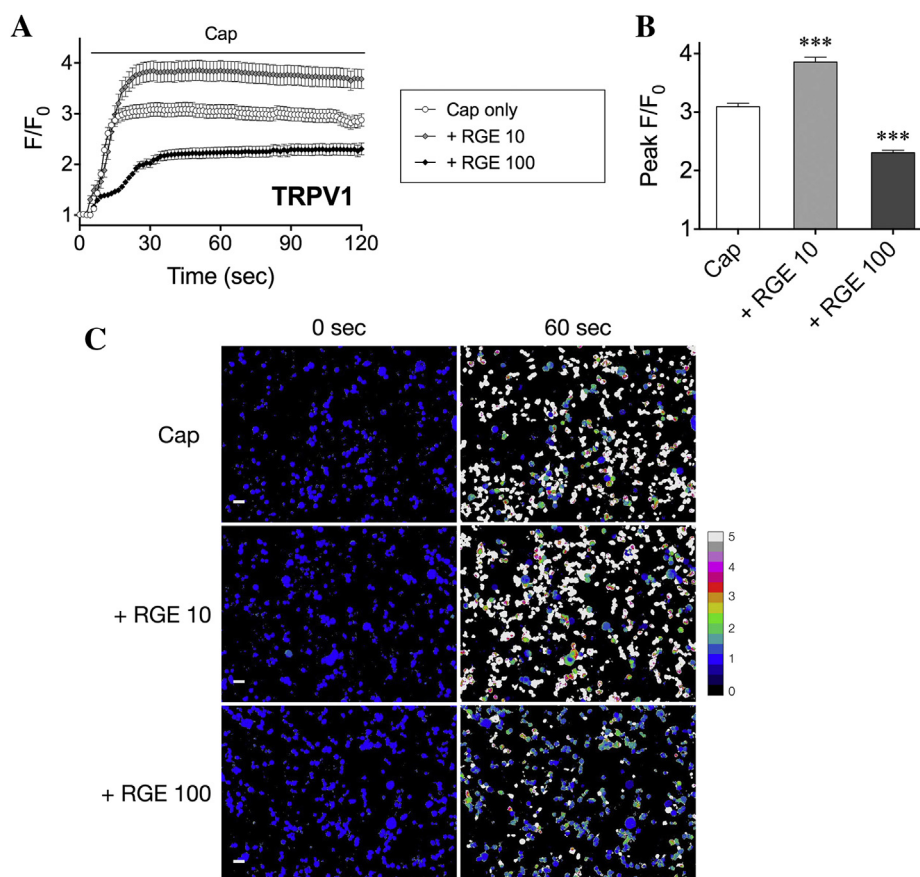
Fig. 4. RGE shows mixed effects on capsaicin-induced Ca^{2+} influx. (A) Traces of Ca^{2+} influx induced by capsaicin in the absence (open circle, $n = 250$) or presence (10 μg/mL: gray, $n = 194$, 100 μg/mL: black, $n = 338$) of RGE for 120 seconds in HEK293T cells expressing TRPV1 alone. (B) Summary of peak Ca^{2+} influx (F/F_0) induced by either capsaicin alone or capsaicin pretreated with RGE. Notice the different effects induced by different RGE concentrations. *** $p < 0.001$, ANOVA with Tukey's *post hoc* test. (C) Representative ratiometric calcium imaging data of capsaicin-induced Ca^{2+} influx before and after treatment of 10 μg/mL or 100 μg/mL RGE at 60 seconds. Scale bar = 20 μm. ANOVA, analysis of variance; RGE, red ginseng extract.

dependence" is also corroborated by Han et al's [25] recent work with TRPV1 knockout mice experiments. They prepared special mice from TRPV1$^{-/-}$ mice, which re-express TRPV1 specifically in itch-specific neurons. When these mice were treated with-capsaicin, scratching behavior was observed but not pain-indicative wiping [25], suggesting that the type of neuron determines the sensation, not the ion channel *per se*.

Although our data clearly demonstrated the antipruritic effect of RGE at sensory neuronal levels, there are several limitations that were not covered in the current study. One would be the identification of key ingredients of RGE that have antipruritic effects at sensory neuronal levels. Although we are currently working on it, there already are good candidate ingredients that need further examination. For example, ginsenoside Rg3 is a good candidate because it reduced the skin severity scores of lesions of the 2-chloro-1,3,5-trinitrobenzene-treated atopic dermatitis mouse model [14]. Rg5 has also been reported to improve chronic dermatitis or psoriasis in a oxazolone-induced mouse ear contact dermatitis model [26]. Therefore, further study will be mandatory in order to determine which ingredients are actually effective in sensory neurons.

The presence of itch that is not related to histamine (or histamine-independent) was recently found. Chloroquine, an antimalaria drug, has been reported to induce histamine-independent itch as a side effect [27]. However, the underlying molecule was not known until recently. It is now known that the responsible molecule is MRGPRA3, which is activated by

chloroquine [28]. In addition, it was also found that MRGPRA3 is functionally linked with TRPA1 ion channel [29]. Considering that TRPA1 is highly expressed in sensory neurons along with TRPV1 [30], further examination of RGE on chloroquine-induced itch will be interesting.

In conclusion, the current study found for the first time that RGE effectively blocks histamine-induced itch in peripheral sensory neurons. We believe that the current results will provide insights and clues with regard to how red ginseng exhibits antipruritic effects at molecular levels.

Conflicts of interest

The authors have declared that there is no conflict of interest.

Acknowledgments

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