Particular ginsenosides have a potential to inhibit calcium influx via H1R/TRPV1 and MrgprA3/TRPA1 pathway that are required in itch transduction

Wook-Joo Lee, Da-Som Choi, Won-Sik Shim

College of Pharmacy, Gachon University, Incheon, Korea

Abstract

Purpose:

Korean red ginseng (the steamed root of *Panax ginseng* C.A. Meyer, family Araliaceae) has been used as a remedy for wide variety of disorders, and recent studies have identified its beneficial use in itch-related diseases such as atopic dermatitis. Although its anti-pruritic effect was mostly revealed by animal scratching tests, its underlying molecular mechanism has never been investigated. Because the itch transduction in peripheral nervous system could be initiated via histamine-dependent (H1R/TRPV1), or -independent (MrgprA3/TRPA1) pathway, the putative inhibitory effect of various ginsenosides were investigated in the present study.

Method:

cDNA combinations of H1R/TRPV1 or MrgprA3/TRPA1 were transiently expressed in human embryonic kidney (HEK) 293T cells, and calcium influx was monitored with Fluo3-AM, a calcium-specific fluorescent dye. To test the inhibitory effect, 11 ginsenosides were pretreated for 10 min, and either histamine (for H1R/TRPV1) or chloroquine (for MrgprA3/TRPA1) was treated and changes were recorded for 1 min.

Result:

It was found that some particular ginsenosides strongly inhibited the calcium influx on both H1R/TRPV1- and MrgprA3/TRPA1-expressing cells even after histamine or chloroquine was treated, suggesting that these ginsenosides may have a potential anti-pruritic activity. Furthermore, it seems that the inhibitory effects of ginsenosides are not mediated by direct blockage of TRPV1, whereas the direct inhibition might take place in case of TRPA1.

Conclusion:

While further in-depth investigation is mandatory, these findings open the possibility to identify which ginsenoside plays a major role in prohibiting the imitation of pruritic signal transduction, either H1R/TRPV1 and/or MrgprA3/TRPA1 pathway, at the peripheral nervous system.

1

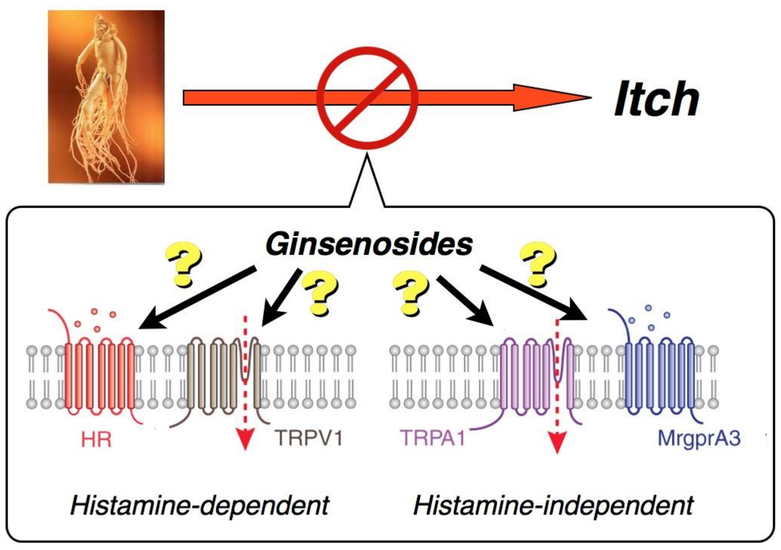

Figure 1. A schematic illustration of the study design. To verify if the anti-pruritic effect of red ginseng has any inhibitory effect on the itch signal transduction at the peripheral level, the two most well-known itch transduction pathway, namely H1R/TRPV1 and MrgprA3/TRPA1 were chosen in the present study. Specifically, the inhibitory effect of various ginsenosides were tested on either H1R/TRPV1- or MrgprA3/TRPA1-expressing HEK293T cells by fluorescent-based calcium imaging assay.

2

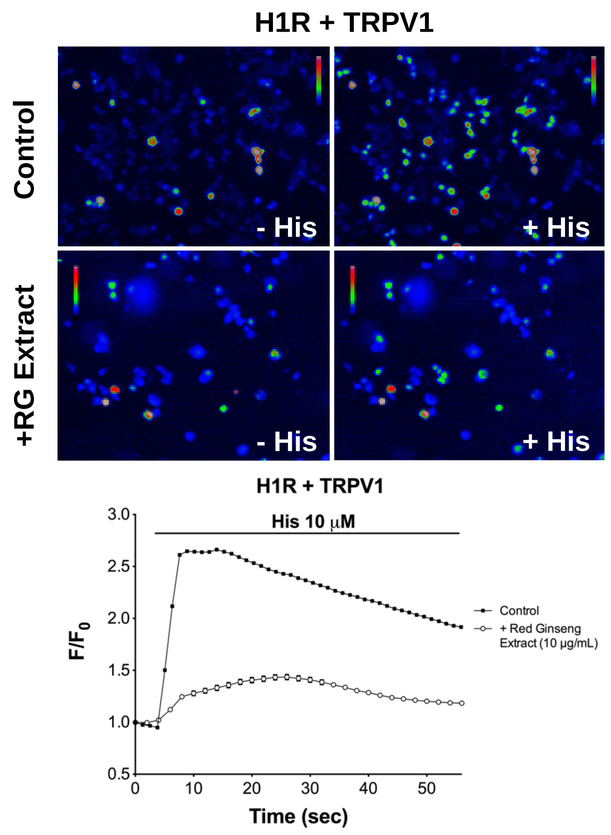

Figure 2. The inhibitory effect of red ginseng extract on H1R/TRPV1 activation by histamine . Pretreatment of red ginseng extract (10 ug/mL) strongly inhibited the histamine (10 uM) response on cells expressing H1R/TRPV1.

3

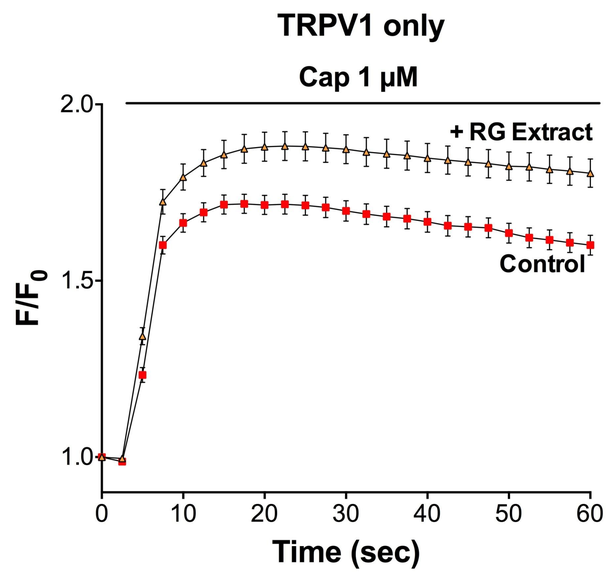

Figure 3. The inhibitory effect of red ginseng extract is not attributable to direct TRPV1 inhibition. When red ginseng extract (10 ug/mL) was pretreated on cells expressing TRPV1 alone, it not only failed to inhibit the capsaicin response on TRPV1, but rather slightly increased the capsaicin response. Therefore, it is expected that the inhibitory effect of red ginseng extract on H1R/TRPV1 is at least not a result of direct inhibition on TRPV1, further implying that red ginseng extract interferes with histamine signal cascade "before" it arrives on TRPV1.

4

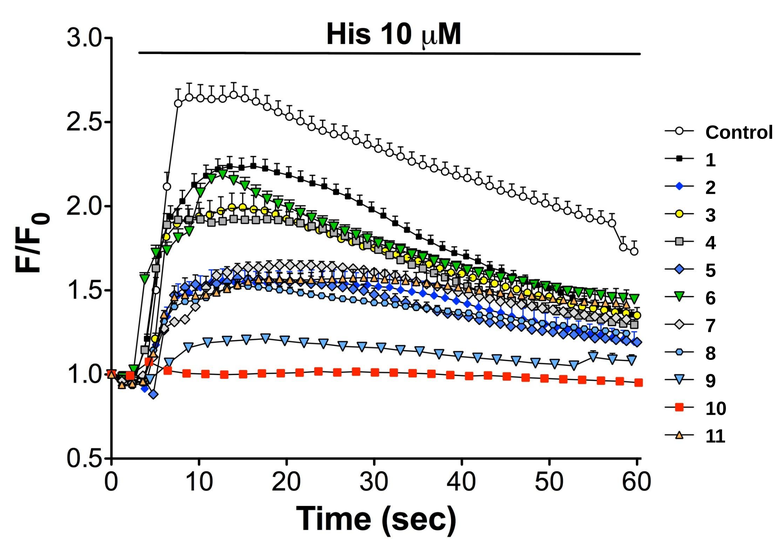

Figure 4. The effect of various ginsenosides on the histamine activation in the H1R/TRPV1 pathway. The inhibitory effect was markedly different among various ginsenosides (1~11, 100 uM), which were pretreated for 10 minutes and histamine (10 uM) was later applied. Notice the strong inhibitory action of ginsenoside 9 and 10 compared to other ginsenosides.

5

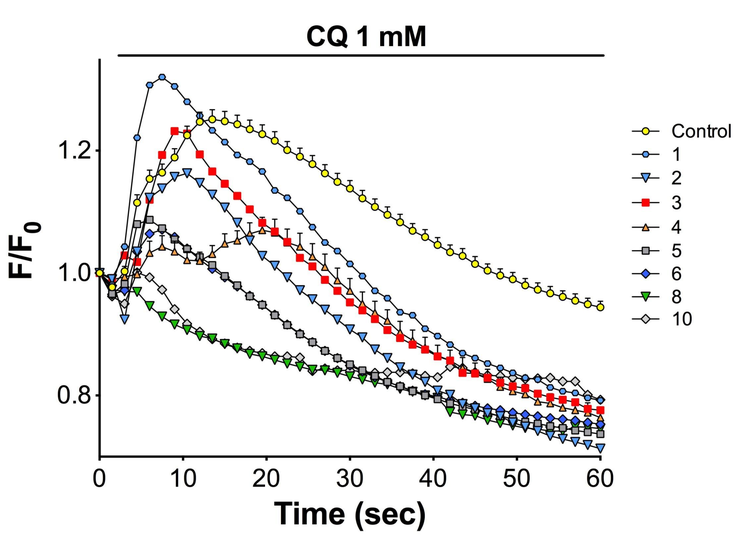

Figure 5. The effect of various ginsenosides on the chloriquine activation in the MrgprA3/TRPA1 pathway. The inhibitory effect was markedly different among various ginsenosides (100 uM), which were pretreated for 10 minutes and chrloquine (1 mM, MrgprA3 agonist) was later applied. The missing ginsenoside 9 and 11 were under experiments at the moment. Ginsenoside 8 and 10 showed the remarkable potency among other ginsenosides.

6

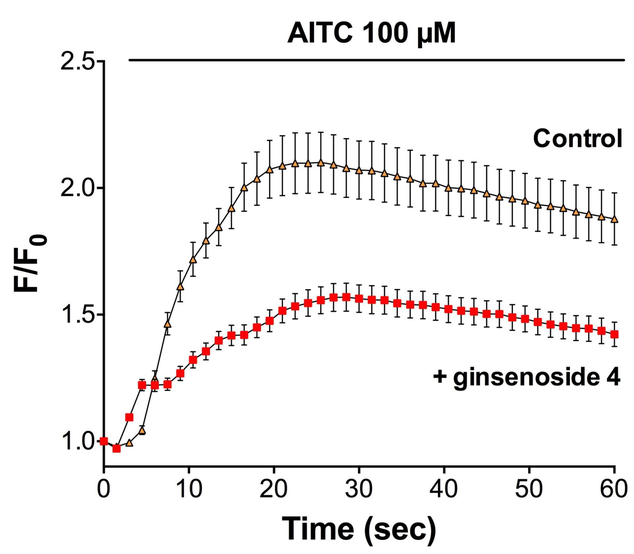

Figure 6 The target of ginsenoside 4 effect might be TRPA1 rather than MrgprA3. An exemplary result of the inhibitory effect of ginsenoside 4 on the AITC (TRPA1 agonist, 100 uM)-induced TRPA1 activity. A inhibitory pattern was similar to that of chloroquine-induced experiments, suggesting that the target of ginsenoside 4 could more likely to be TRPA1 rather than MrgprA3.