JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Transient receptor potential vanilloid-1 participates in the inhibitory effect of ginsenoside Rg1 on capsaicin-induced interleukin-8 and prostaglandin E_2 production in HaCaT cells

Jin Huang[a], Li Ding[b], Duo Shi[b], Jin-hong Hu[a], Quan-gang Zhu[a], Shen Gao[a] and Lei Qiu[b]

[a]Department of Pharmacy, Changhai Hospital, and [b]Department of Biochemical Pharmacy, College of Pharmacy, Second Military Medical University, Shanghai, China

Keywords
ginsenoside Rg1; interleukin-8; keratinocyte; prostaglandin E_2; transient receptor potential vanilloid-1

Correspondence
Lei Qiu, Department of Biochemical Pharmacy, College of Pharmacy, Second Military Medical University, Shanghai, 200433, China.
E-mail: qiulei70399@yahoo.com.cn

Received June 7, 2011
Accepted September 22, 2011

doi: 10.1111/j.2042-7158.2011.01392.x

Abstract

Objectives Ginsenoside Rg1 (GRg1), one of the major active constituents of *Panax notoginseng*, has shown anti-inflammatory and antinocioceptic activity, but its role in keratinocytes needs further study. We have examined the inhibitory effect of GRg1 on transient receptor potential vanilloid-1 (TRPV1) activation in keratinocyte HaCaT cells and explored its involved mechanism.

Methods HEK 293T cells over-expressing exogenous TRPV1 were constructed and named HEK 293T-TRPV1 cells. The effects of GRg1 on production of interleukin-8 (IL-8) and prostaglandin E_2 (PGE_2), calcium influx, the expression of cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB) transcriptional activity in HEK 293T-TRPV1 and HaCaT cells were examined by ELISA, Fluo 3-AM fluorescence probe, Western blot and Dual-Luciferase Reporter Assay, respectively.

Key findings The results showed that GRg1 blocked intracellular calcium by both capsaicin and proton activation in a TRPV1-dependent manner. Furthermore, GRg1 inhibited the expression of COX-2 and NF-κB transcriptional activity induced by capsaicin in keratinocytes. The inhibitory effect of GRg1 was similar to capsazepine, an antagonist of TRPV1. More importantly, GRg1 dose-dependently inhibited capsaicin-induced PGE_2 and IL-8 secretion in HaCaT cells and HEK 293T-TRPV1 cells.

Conclusions These data showed that GRg1 could inhibit TRPV1 mediated responses in HaCaT cells, indicating that GRg1 acted as a TRPV1 antagonist.

Introduction

Panax notoginseng is a well-known medicinal herb in Asia, and has been widely used in Chinese compound formulations to treat skin disorders. Ginsenoside Rg1 (GRg1) (Figure 1) is one of the major bioactive components of *P. notoginseng*, and shows a variety of pharmacological activity on coronary heart disease and cancer.[1,2] Previous studies have demonstrated that GRg1 inhibited the production of pro-inflammatory factors and showed immunoregulatory activity in some immunological diseases.[3,4] However, the effect of GRg1 on skin disorders and its possible mechanism has remained unclear.

Transient receptor potential vanilloid-1 (TRPV1) is first identified in sensory neurons, and is involved in nocioception and inflammation.[5–8] Recently, it has been demonstrated that TRPV1 contributed to the release of inflammatory factors in non-neuronal cells, including skin keratinocytes, lung cells and primary cultured epithelial cells.[9–11] In the skin of rats and humans, immunohistochemical analysis has indicated that TRPV1 is located in a neurochemically heterogeneous population of small-diameter primary afferent fibres and with small-diameter nerve fibres.[5,12] These data reveal that TRPV1 is clearly involved in neurogenic inflammation of human skin and can be downregulated by anti-inflammatory agents such as topical calcineurin inhibitors.[5,13,14]Thus far, a role for TRPV1 has been observed on keratinocyte proliferation, differentiation, and apoptosis, and probably the release of cytokine mediators from keratinocytes.[9,13]

Keratinocytes act as the primary barrier in skin and alert the host to danger via the release of some cytokines and chemokines, such as interleukin-6 (IL-6), IL-8 and prostaglandins (PEG_2).[15–17] Furthermore, the TRPV1 receptor has been shown to be a nonselective cationic channel with high

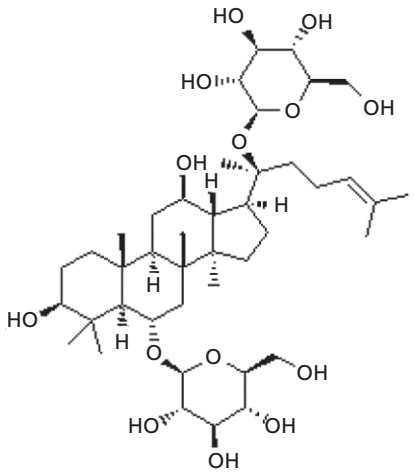

Figure 1 Structure formula of ginsenoside Rg1.

permeability to calcium. In keratinocytes, it could be activated by both endogenous and exogenous factors, such as high temperature, low pH (proton), conditions that occur during tissue injury, or capsaicin. Therefore, the TRPV1 pathway has been a promising target for the development of anti-inflammatory and analgesic drugs in the treatment of skin disorders.[18,19]

Previous studies have found that ginsenosides could modulate the functions of receptors and ion channels, including N-methyl-D-aspartate (NMDA) receptor and Ca^{2+} channel on neurons.[20–22] As one of the important substances of ginsenosides, GRg1 could regulate Ca^{2+} influx in neuronal cells. All that evidence indicates the anti-inflammatory capacity of GRg1, and raises the possibility that GRg1 may be a candidate drug for the treatment of skin disorders. Therefore, in this study, the inhibitory effects of GRg1 on TRPV1 activation in HaCaT cells have been investigated, including its possible pathway. We showed that GRg1 acted as an anti-inflammatory substance through the TRPV1-dependent pathway, which has potential in the clinical treatment of skin diseases.

Materials and Methods

Chemical and reagents

GRg1 (purity > 99%) was purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA). The goat anti-human cyclooxygenase-2 (COX-2) polyclonal antibody and anti-β-actin antibody were purchased from Santa Cruz Biotechnology (CA, USA). The horseradish peroxidase-coupled secondary antibody was bought from Immunology Consultants Laboratory (Newberg, OR, USA). Fluo 3-AM was acquired from DojinDo Laboratories (Kumamoto, Japan). Enzyme-linked immunosorbent assay (ELISA) kits for IL-8 and PGE_2 were purchased from R&D Systems (Minneapolis, MN, USA). Dual-Luciferase Reporter system was purchased from Promega (Madison, WI, USA).

Cell culture

HEK 293T-TRPV1 cells and mock cells were constructed as described by Huang *et al*.[23] HaCaT cells and HEK 293T-TRPV1 cells were cultured using Dulbecco's modified essential medium (DMEM) containing 10% (v/v) fetal bovine serum (Invitrogen, Carlsbad, CA, USA) and penicillin/streptomycin in 5% CO_2 at 37°C.

Detection of intracellular Ca^{2+}

Cells (1×10^6/well) were cultured in calcium-containing DMEM overnight and then pretreated with GRg1 (0~20 μM) or capsazepine (a TRPV1 antagonist, 3 μM) for 30 min. Fluo-3 AM (10 μM; DojinDo Laboratories, Japan) was loaded into HEK 293T-TRPV1 cells or HaCaT cells for 15 min at 37°C. Cells were then treated with capsaicin, or supplemented with 30 mM HEPES, 30 mM MES-buffer, and 0.1 mg/ml bovine serum albumin adjusted to pH 4.1 with HCl (final assay pH 5) and left for an additional 2 min before medium replacement. Fluorescence changes were examined by flow cytometry. The fluorescence of Fluo-3 AM was excited at 488 nm and measured via a 525-nm filter. Photomultiplier tube voltages and gains were set to optimize the dynamic range of the signal. The fluorescence intensity was quantified for 10 000 individual cells. The experiments were repeated three times and mean ± SD were reported.

Cyclooxygenase-2 protein expression

HaCaT cells (2×10^6 cells/well) were seeded in 6-well plates and cultured overnight, then pretreated with GRg1 (1, 10 or 100 μM) or capsazepine (3 μM) for 30 min followed by stimulation with capsaicin (10 μM) for 6 h. Cells were then washed twice with ice-cold phosphate-buffered saline and lysed. Samples were quantified with the BCA Protein Assay Reagent kit (Pierce, Rockford, IL, USA) and were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% polyacrylamine gel. Proteins were electrotransferred onto nitrocellulose filter membranes (Invitrogen, Carlsbad, CA, USA). Membranes were incubated with goat anti-human COX-2 polyclonal antibody (1 : 1000 dilution; Santa Cruz Biotech) and anti-β-actin (Santa Cruz Biotech), followed by a rabbit anti-goat horseradish (HRP) secondary antibody (1 : 8000 dilution; Immunology Consultants Laboratory). The intensity of the bands was directly quantified by Image QuaNT software (Molecular Dynamics/Amersham Biosciences, Piscataway, NJ, USA). Arbitrary units represent the ratio between COX-2 and β-actin volumes.

Detection of nuclear factor-κB transcriptional activity

Dual-Luciferase Reporter Assay was used to detect the nuclear factor-κB (NF-κB) transcriptional activity. Cells were co-transfected with a mixture of pGL3.5Xκb-luciferase (Promega, Madison, WI, USA) and pRL-TK-Renilla-luciferase (Promega, Madison, WI, USA) by lipofectamine 2000 reagent (Promega, Madison, WI, USA). After 24 h, cells were pretreated with 10 μM GRg1 or 3 μM capsazepine for 30 min and then treated with 10 μM capsaicin for 6 h. NF-κB luciferase activity was measured according to the manufacturer's instructions. Data were normalized for NF-κB activity by dividing firefly luciferase activity with that of Renilla luciferase.

Measurement of interleukin-8 and prostaglandin E_2 levels by ELISA

HaCaT cells (2×10^6 cells/well) were seeded in 6-well plates and cultured overnight. The cells were then pretreated with GRg1 (1, 10 or 100 μM) or capsazepine (3 μM) for 30 min followed by stimulation with capsaicin (10 μM). After 8 or 24 h the cell supernatants were collected and IL-8 or PGE_2 levels were determined by ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). The same method was performed with cell supernatants from HEK 293T mock and HEK 293T-TRPV1 cells, which were pretreated with GRg1 or capsazepine for 30 min followed by stimulation with capsaicin (10 μM) for 24 h. The absorbance was read at the wavelength of 450 nm with a Wellscan MK3 microplate reader (Labsystems Dragon, Helsinki, Finland). Results were presented as mean ± SD values of three independent experiments.

Statistical analysis

All data were presented as mean ± SD. Data were analysed by Student's *t*-test and one-way analysis of variance. A *P*-value less than 0.05 was considered statistically significant.

Results

GRg1 inhibited calcium influx in TRPV1 expressing cells

To explore the effect of GRg1 on the TRPV1 activation, the intracellular calcium levels were measured in HaCaT cells, HEK 293T mock cells and HEK 293T-TRPV1 cells. The results showed that capsaicin significantly increased intracellular calcium in HaCaT cells and HEK 293T-TRPV1 cells in a dose-dependent manner, but had no effect in HEK 293T mock cells (Figure 2). GRg1 alone had no effect on the calcium influx in HaCaT cells and HEK 293T-TRPV1 cells, however, GRg1 (10 μM) significantly decreased capsaicin-induced calcium influx in HaCaT cells and HEK 293T-TRPV1 cells by 33.95% ($P < 0.05$) and 41.67% ($P < 0.05$), respectively, which showed similar inhibitory effects as capsazepine (3 μM).

For proton activation, the effect of GRg1 was only measured in HaCaT cells, because nontransfected HEK 293T cells showed proton-activated currents (data not shown). The results of proton activation were similar with that of capsaicin activation in HaCaT cells (Figure 2d). GRg1 (10 μM) significantly decreased proton-activated calcium influx in HaCaT cells by 19.76% ($P < 0.05$). These results indicated that TRPV1 contributed to the inhibitory effect of GRg1 on both capsaicin and proton-induced intracellular calcium.

Inhibition of GRg1 on cyclooxygenase-2 expression

To further determine the inhibitory effect of GRg1 on the TRPV1-mediated responses, we detected the COX-2 expression in HaCaT cells. Cells were pretreated with GRg1 or capsazepine for 30 min followed by stimulation with capsaicin (10 μM) for 6 h and expression of COX-2 was examined. The results showed that capsaicin significantly induced the expression of COX-2 in HaCaT cells. This effect was substantially attenuated by a TRPV1 antagonist, capsazepine. More importantly, HaCaT cells pretreated with increasing concentrations of GRg1 resulted in a dose-dependent inhibition in COX-2 expression (Figure 3).

GRg1 inhibited capsaicin-induced nuclear factor-κB transcriptional activity

The transcriptional factor NF-κB plays an important role in regulating pro-inflammatory genes, including PGE_2 and IL-8. Capsaicin (10 μM) significantly increased the NF-κB transcription activity in HaCaT cells ($P < 0.001$) and in HEK 293T-TRPV1 cells ($P < 0.001$), but had no remarkable effect in HEK 293T mock cells. After HaCaT and HEK 293T-TRPV1 cells were pretreated with GRg1 (10 μM), the NF-κB transcription activity induced by capsaicin was significantly decreased ($P < 0.05$, Figure 4b). Similar results were obtained when cells were pretreated with capsazepine (3 μM) ($P < 0.05$). These data suggested that GRg1 could inhibit NF-κB activity induced by capsaicin in a TRPV1-dependent pathway.

GRg1 inhibited capsaicin-induced prostaglandin E_2 and interleukin-8 secretion

Inflammation of the skin is coupled with the release of various pro-inflammatory mediators. In this study, we determined the levels of IL-8 and PGE_2 in the culture supernatants by ELISA. As shown in Figure 5a and b, the levels of IL-8 and PGE_2 released from HaCaT cells were significantly increased

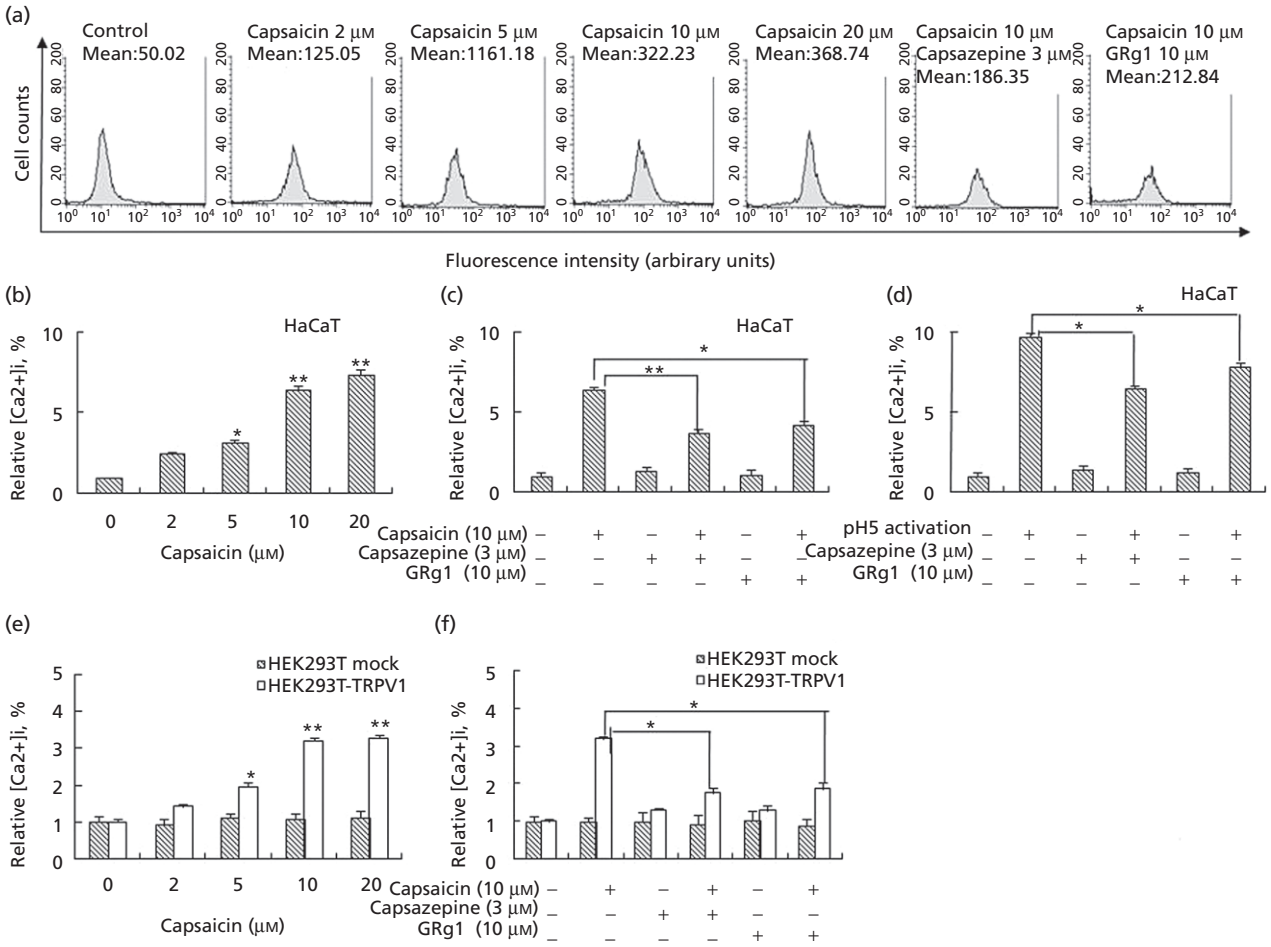

Figure 2 Ginsenoside Rg1 inhibited capsaicin-induced intracellular calcium. Relative $[Ca^{2+}]i$ was measured by fluorescence intensity of Fluo-3 AM (a calcium indicator dye) via flow cytometry. Experiments were performed three times with similar results. $^{**}P < 0.001$, $^{*}P < 0.05$.

after treatment with 10 μM capsaicin ($P < 0.05$) for 8 or 24 h. After pretreatment with various concentrations of GRg1, the production of PGE_2 and IL-8 induced by capsaicin were reduced in a dose-dependent manner. For the production of PGE_2, the inhibitory rates of 1, 10 and 100 μM GRg1 were 21.80% ($P < 0.05$), 29.46% ($P < 0.001$) and 37.35% ($P < 0.001$) in HaCaT cells after stimulation for 24 h, respectively. The levels of IL-8 were decreased by 15.71%, 27.06% and 30.15% ($P < 0.001$), respectively. In addition, the IL-8 and PGE_2 levels were detected in the supernatant of HEK 293T-TRPV1 and mock cells. The results showed that the IL-8 and PGE_2 levels in HEK 293T-TRPV1 supernatant, but not in HEK 293T mock supernatant, were significantly increased after being treated with 10 μM capsaicin ($P < 0.05$)(Figure 5c and d). The release of IL-8 and PGE_2 was inhibited by GRg1 in a dose-dependent manner in HEK 293T-TRPV1 cells. These data suggested that the induction of capsaicin on the release of IL-8 and PGE_2 was TRPV1-dependent and the inhibitory role of GRg1 was mediated by TRPV1.

Discussion

The presence of functional TRPV1 receptors in keratinocytes has generated interest in skin–nerve crosstalk under physiological and pathological conditions.[5] It has been speculated that keratinocytes expressing TRPV1 respond to noxious environmental or inflammatory stimuli and produce interleukins that, in turn, activate TRPV1-expressing sensory nerves. Given the essential role of TRPV1 in the inflammatory response, it is hardly surprising that it is a hot point to developing TRPV1 antagonists. These antagonists with different chemical structures have been reported to block TRPV1 channel activation. Antagonists have been classified into different profiles based on their ability to differentially modulate distinct modes of TRPV1 activation, including capsaicin, pH 5 and heat activation.[24,25] In this study, we have shown that GRg1, one of the major bioactive components of *P. notoginseng*, strongly inhibited capsaicin and proton-induced biological responses in a TRPV1-dependent manner.

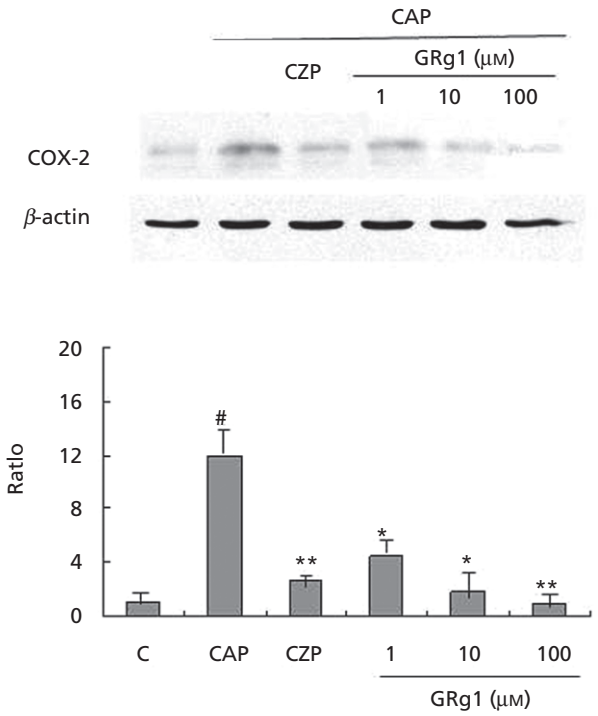

Figure 3 Ginsenoside Rg1 inhibited cyclooxygenase-2 expression in HaCaT cells. Cells were pretreated with ginsenoside Rg1 (GRg1) or capsazepine (CZP) for 30 min followed by stimulation with capsaicin (CAP; 10 μM) for 6 h and expression of cyclooxygenase-2 (COX-2) was examined by Western blot. Protein sample was separated using 10% SDS-polyacrylamide gel electrophoresis, and COX-2-immunoreactivity was determined using polyclonal antibody. C, control.. $n = 3$, $^{**}P < 0.001$, $^{*}P < 0.05$.

It suggests that GRg1 has potential in clinical treatment of skin diseases as a potent inhibitor of the TRPV1 receptor.

GRg1 is considered to be the principal active constituent of natural herbal compounds isolated from *P. notoginseng*, which are used widely to treat skin diseases, based on their anti-inflammatory activities.[2,26,27] Some evidence indicated that ginsenosides could directly block capsaicin-activated channels, resulting in attenuation of the currents in rat sensory neurons.[28] It strongly suggested that the attenuation by ginsenosides of capsaicin-induced pain behaviour arose from their interaction with TRPV1 channel function. Previously, we had reported that GRb1 inhibited IL-8 and PGE_2 production via TRPV1-dependent calcium influx in HaCaT cells.[23] Both GRg1 and GRb1 are the important constituents of ginsenosides, but unlike GRb1, GRg1 belongs to the panaxatriol group of ginsenosides.[29] Although their chemical structures are different, both of them showed the inhibitory effect on TRPV1 activation.

As we know, increasing calcium influx contributes to signal pathway activation and several pathological conditions. TRPV1 is a nonselective cationic channel that conducts calcium ions and capsaicin-induced activation of the epidermal TRPV1 results in a similar calcium conductance.[30,31] COX-2 expression by epithelial cells requires an increase in intracellular calcium and this expression can be mimicked with calcium ionophores or inhibited by removal of extracellular calcium.[32,33] Thus, the activation of TRPV1 and the subsequent elevation of intracellular calcium are of primary importance in the capsaicin-mediated production of pro-inflammatory mediators. On the other hand, Ca^{2+} influx may serve as a messenger to activate NF-κB directly or indirectly, resulting in the induction of various pro-inflammatory genes.[34]

In this study, the inhibitory effects of GRg1 on the TRPV1-mediated responses have been examined in keratinocytes. The results showed that GRg1 significantly downregulated capsaicin-induced IL-8 and PGE_2 production in HaCaT cells and HEK 293T-TRPV1 cells, but not in HEK 293T mock cells. These data suggested that GRg1 inhibited capsaicin-induced activation of keratinocytes through the TRPV1 pathway. As shown in Figure 5, although capsazepine significantly inhibited Ca^{2+} and NF-κB pathway, its effect on IL-8 production was partial. These findings raise the question of whether the release of pro-inflammatory mediators was regulated by other factors besides the NF-κB pathway.

Conclusions

Taken together, we have provided evidence that the inhibitory role of GRg1 in several capsaicin-induced biological responses might have been mediated by TRPV1: the stimulatory effects of capsaicin on COX-2 expression, NF-κB activation and IL-8 and PGE_2 production were dependent on TRPV1 expression; capsazepine, a TRPV1 antagonist, significantly inhibited TRPV1-mediated responses; and GRg1 showed similar inhibitory effects as capsazepine only in TRPV1-positive cells, indicating that GRg1 acted as a TRPV1 antagonist.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by the Scientific Research Fund of Shanghai Health Bureau (2009109).

Acknowledgements

The authors wish to thank Prof. Dr. Zoltán Sándor for kindly providing plasmid and Prof. Jun Gu (Department of Dermatology, Changhai Hospital, SMMU, Shanghai, China) for providing HaCaT cells.

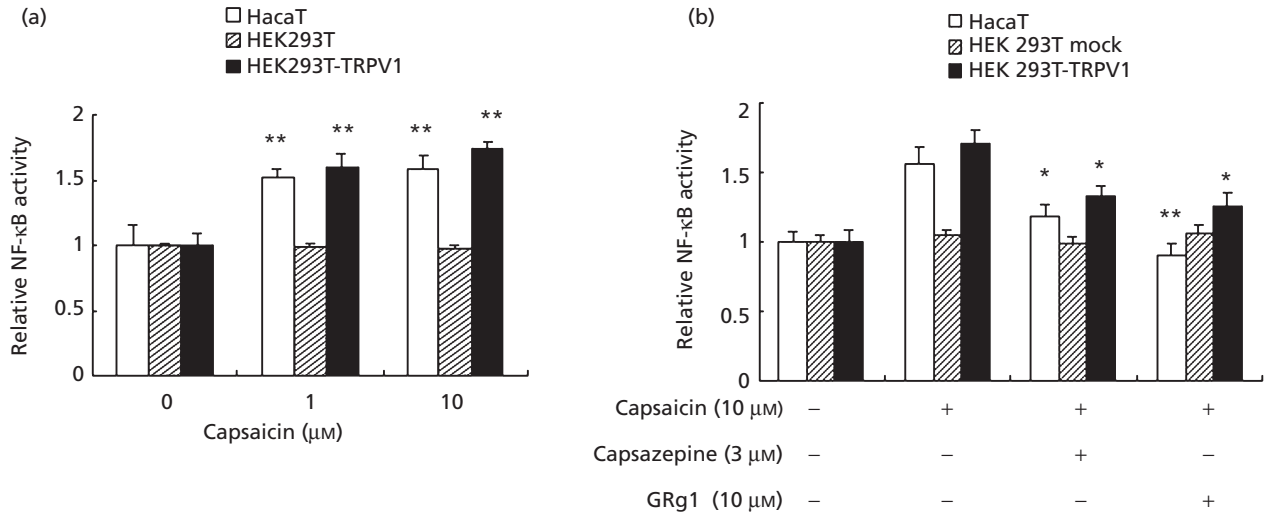

Figure 4 Ginsenoside Rg1 inhibited nuclear factor-κB transcriptional activity. HaCaT cells, HEK 293T mock cells and HEK 293T-TRPV1 cells were co-transfected with a mixture of pGL3.5XκB-luciferase and pRL-TK-Renilla-luciferase using lipofectamine reagent. Twenty-four hours later, they were treated with the indicated concentrations of capsaicin for 6 h and then lysed. Nuclear factor-κB (NF-κB) activity was measured using the Dual-Luciferase Reporter Assay System and normalized by Renilla-luciferase activity. Ginsenoside Rg1, GRg1. Data were shown as mean ± SD values from three independent experiments. $n = 5$; $^{**}P < 0.001$, $^{*}P < 0.05$.

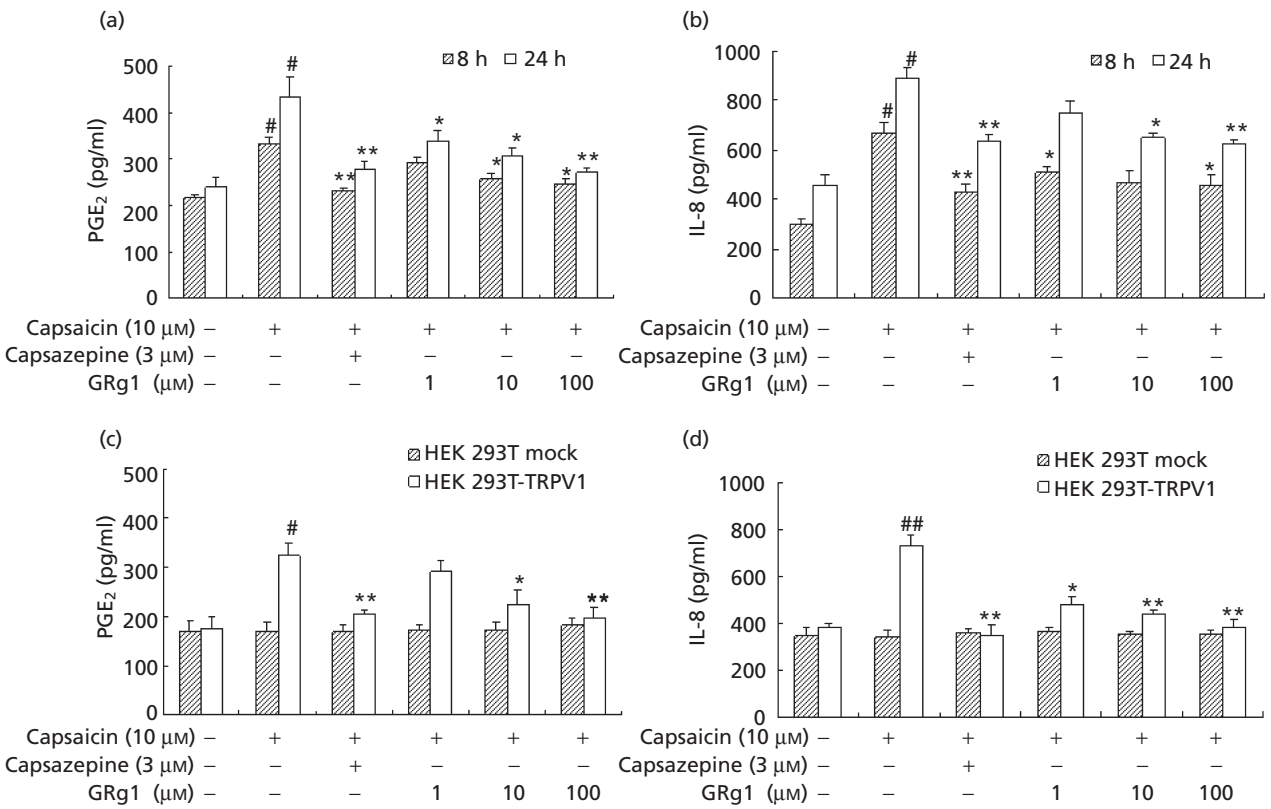

Figure 5 Ginsenoside Rg1 inhibited the production of prostaglandin E_2 and interleukin-8. (a–b) The release of prostaglandin E_2 (PGE_2) and interleukin-8 (IL-8) was detected from the supernatant of HaCaT cells stimulated by capsaicin for 8 or 24 h. Ginsenoside Rg1, GRg1. (c–d) Data were detected from the supernatant of HEK 293T mock and HEK 293T-TRPV1 cells stimulated by capsaicin for 24 h. Results were shown as mean ± SD values from three independent experiments. $n = 5$; $^{**}P < 0.001$, $^{*}P < 0.05$ compared with capsaicin treatment; $^{\#\#}P < 0.001$, $^{\#}P < 0.05$ compared with no treatment.

References

1. Li L *et al*. High-performance liquid chromatographic assay for the active saponins from Panax notoginseng in rat tissues. *Biomed Chromatogr* 2006; 20: 327–335.
2. Ng TB. Pharmacological activity of sanchi ginseng (*Panax notoginseng*). *J Pharm Pharmacol* 2006; 58: 1007–1019.
3. Surh YJ *et al*. Effects of selected ginsenosides on phorbol ester-induced expression of cyclooxygenase-2 and activation of NF-kappaB and ERK1/2 in mouse skin. *Ann N Y Acad Sci* 2002; 973: 396–401.
4. Wang Y *et al*. Metabolism of ginsenoside Rg1 by intestinal bacteria. II. Immunological activity of ginsenoside Rg1 and Rh1. *Acta Pharmacol Sin* 2000; 21: 792–796.
5. Stander S *et al*. Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures. *Exp Dermatol* 2004; 13: 129–139.
6. Caterina MJ *et al*. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997; 389: 816–824.
7. Hayes P *et al*. Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. *Pain* 2000; 88: 205–215.
8. Davis JB *et al*. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 2000; 405: 183–187.
9. Southall MD *et al*. Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes. *J Pharmacol Exp Ther* 2003; 304: 217–222.
10. Seki N *et al*. Capsaicin induces the production of IL-6 in human upper respiratory epithelial cells. *Life Sci* 2007; 80: 1592–1597.
11. Lee LY *et al*. Role of TRPV1 in inflammation-induced airway hypersensitivity. *Curr Opin Pharmacol* 2009; 9: 243–249.
12. Bodo E *et al*. Vanilloid receptor-1 (VR1) is widely expressed on various epithelial and mesenchymal cell types of human skin. *J Invest Dermatol* 2004; 123: 410–413.
13. Bodo E *et al*. A hot new twist to hair biology: involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control. *Am J Pathol* 2005; 166: 985–998.
14. Stander S *et al*. Neurophysiology of pruritus: cutaneous elicitation of itch. *Arch Dermatol* 2003; 139: 1463–1470.
15. Alappatt C *et al*. Acute keratinocyte damage stimulates platelet-activating factor production. *Arch Dermatol Res* 2000; 292: 256–259.
16. Kemeny L *et al*. The interleukin-8 receptor: a potential target for antipsoriatic therapy? *Eur J Pharmacol* 1994; 258: 269–272.
17. Kupper TS. The activated keratinocyte: a model for inducible cytokine production by non-bone marrow-derived cells in cutaneous inflammatory and immune responses. *J Invest Dermatol* 1990; 94: 146S–150S.
18. Gunthorpe MJ *et al*. Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. *Drug Discov Today* 2009; 14: 56–67.
19. Khairatkar-Joshi N *et al*. TRPV1 antagonists: the challenges for therapeutic targeting. *Trends Mol Med* 2009; 15: 14–22.
20. Lee JH *et al*. Protective effect of ginsenosides, active ingredients of Panax ginseng, on kainic acid-induced neurotoxicity in rat hippocampus. *Neurosci Lett* 2002; 325: 129–133.
21. Lee E *et al*. 20(S)-ginsenoside Rh2, a newly identified active ingredient of ginseng, inhibits NMDA receptors in cultured rat hippocampal neurons. *Eur J Pharmacol* 2006; 536: 69–77.
22. Rhim H *et al*. Ginseng and ginsenoside Rg3, a newly identified active ingredient of ginseng, modulate Ca2+ channel currents in rat sensory neurons. *Eur J Pharmacol* 2002; 436: 151–158.
23. Huang J *et al*. Ginsenoside Rb1 and paeoniflorin inhibit transient receptor potential vanilloid-1-activated IL-8 and PGE production in a human keratinocyte cell line HaCaT. *Int Immunopharmacol* 2010; 10: 1279–1283.
24. Gavva NR *et al*. AMG 9810 [(E)-3-(4-t-butylphenyl)-N-(2,3-dihydroben zo[b][1,4] dioxin-6-yl)acrylamide], a novel vanilloid receptor 1 (TRPV1) antagonist with antihyperalgesic properties. *J Pharmacol Exp Ther* 2005; 313: 474–484.
25. Lehto SG *et al*. Antihyperalgesic effects of (R,E)-N-(2-hydroxy-2,3-dihydro-1H-inden-4-yl)-3-(2-(piperidin-1-yl)-4-(tri fluoromethyl)phenyl)-acrylamide (AMG8562), a novel transient receptor potential vanilloid type 1 modulator that does not cause hyperthermia in rats. *J Pharmacol Exp Ther* 2008; 326: 218–229.
26. Kim HA *et al*. Anti-arthritic effect of ginsenoside Rb1 on collagen induced arthritis in mice. *Int Immunopharmacol* 2007; 7: 1286–1291.
27. Kawahira K *et al*. Effects of ginsenoside Rb1 at low doses on histamine, substance P, and monocyte chemoattractant protein 1 in the burn wound areas during the process of acute burn wound repair. *J Ethnopharmacol* 2008; 117: 278–284.
28. Hahn J *et al*. Ginsenosides inhibit capsaicin-activated channel in rat sensory neurons. *Neurosci Lett* 2000; 287: 45–48.
29. Harkey MR *et al*. Variability in commercial ginseng products: an analysis of 25 preparations. *Am J Clin Nutr* 2001; 73: 1101–1106.
30. Oh U *et al*. Capsaicin activates a nonselective cation channel in cultured neonatal rat dorsal root ganglion neurons. *J Neurosci* 1996; 16: 1659–1667.
31. Nagy I *et al*. Similarities and differences between the responses of rat sensory neurons to noxious heat and capsaicin. *J Neurosci* 1999; 19: 10647–10655.
32. Guo YS *et al*. Activator protein-1 transcription factor mediates bombesin-stimulated cyclooxygenase-2 expression in intestinal epithelial cells. *J Biol Chem* 2001; 276: 22941–22947.
33. Bazan HE *et al*. Platelet-activating factor induces cyclooxygenase-2 gene expression in corneal epithelium. Requirement of calcium in the signal transduction pathway. *Invest Ophthalmol Vis Sci* 1997; 38: 2492–2501.
34. Kaltschmidt B *et al*. Signaling via NF-kappaB in the nervous system. *Biochim Biophys Acta* 2005; 1745: 287–299.