

Quercetin inhibits histamine-induced calcium influx in human keratinocyte via histamine H4 receptors

Chung-Chi Yang^a, Yen-Ling Hung^b, Hsin-Ju Li^b, Ya-Fan Lin^{c,d}, Su-Jane Wang^e, Der-Chen Chang^f, Chi-Ming Pu^{e,g}, Chi-Feng Hung^{b,c,e,h,*}

^a Division of Cardiovascular Medicine, Taoyuan Armed Forces General Hospital, Taoyuan City, Taiwan

^b Graduate Institute of Biomedical and Pharmaceutical Science, Fu Jen Catholic University, New Taipei City, Taiwan

^c Department of Fragrance and Cosmetic Science, Kaohsiung Medical University, Kaohsiung, Taiwan

^d Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung, Taiwan

^e School of Medicine, Fu Jen Catholic University, Xinzhuang, New Taipei City, Taiwan

^f Department of Mathematics and Statistics and Department of Computer Science, Georgetown University, Washington, DC 20057, USA

^g Division of Plastic Surgery, Department of Surgery, Cathay General Hospital, Taipei 10630, Taiwan

^h Program in Pharmaceutical Biotechnology, Fu Jen Catholic University, New Taipei City, Taiwan

ARTICLE INFO

Keywords:

Quercetin

Histamine

Calcium influx

TRPV1

Histamine 4 receptor

ABSTRACT

Histamine is released from mast cells when tissues are inflamed or stimulated by allergens. Activation of histamine receptors and calcium influx via TRPV1 could be related to histamine-induced itch and skin inflammation. Quercetin is known to have anti-inflammatory and anti-itching effects. This study aims to understand whether quercetin can directly affect histamine-induced calcium influx in human keratinocyte. In it, we investigated quercetin, which acts on histamine-induced intracellular free calcium ($[Ca^{2+}]_i$) elevation in human keratinocyte. Changes in $[Ca^{2+}]_i$ were measured using spectrofluorometry and confocal Imaging. We detected the expression of IL-8 after treatment of quercetin using qRT-PCR and evaluated its anti-itching effect in BALB/c mice. We also performed a docking study to estimate the binding affinity of quercetin to H4 receptors. We found that quercetin pretreatment decreased histamine-induced $[Ca^{2+}]_i$ elevation in a concentration-dependent manner. The inhibitory effect of quercetin on histamine-induced $[Ca^{2+}]_i$ elevation was blocked by JNJ7777120, a selective H₄ antagonist, as well as by U73122, a PLC inhibitor, and by GF109203X, a PKC inhibitor. We also found that H₄ agonist (4-methylhistamine)-induced $[Ca^{2+}]_i$ elevation could be inhibited by quercetin. Moreover, the selective TRPV1 blocker capsazepine significantly suppressed the quercetin-mediated inhibition of histamine-induced $[Ca^{2+}]_i$ elevation, whereas the TRPV4 blocker GSK2193874 had no effect. Last, quercetin decreased histamine and H₄ agonist-induced IL-8 expression in keratinocyte and inhibited the scratching behavior-induced compound 48/80 in BALB/c mice. The molecular docking study also showed that quercetin exhibited high binding affinities with H4 receptors (autodock scores for H4 = −8.7 kcal/mol). These data suggest that quercetin could decrease histamine 4 receptor-induced calcium influx through the TRPV1 channel and could provide a molecular mechanism of quercetin in anti-itching, anti-inflammatory, and unpleasant sensations.

Abbreviations: 4-MH, 4-Methylhistamine; DNCB, 2,4-Dinitrochlorobenzene; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; DMSO, Dimethyl sulfoxide; AD, Atopic dermatitis; DAG, Diacylglycerol; IP₃, Inositol 1,4,5-trisphosphate; MMP, Matrix metalloproteinase; PLC, Phospholipase C; TSLP, Thymic stromal lymphopoietic protein; TRPV1, Transient receptor potential vanilloid-1.

* Corresponding author.

E-mail addresses: t220979@ms16.hinet.net, t220979@gmail.com (C.-C. Yang), 409466019@mail.fju.edu.tw, 405470127@mail.fju.edu.tw (Y.-L. Hung), sakumanatsumi@hotmail.com (H.-J. Li), med0003@mail.fju.edu.tw, yafan@kmu.edu.tw (Y.-F. Lin), 049700@mail.fju.edu.tw (S.-J. Wang), 409466019@mail.fju.edu.tw, chang@georgetown.edu (D.-C. Chang), 054317@gapp.fju.edu.tw, pkman9335@msn.com (C.-M. Pu), skin@mail.fju.edu.tw (C.-F. Hung).

<https://doi.org/10.1016/j.intimp.2021.107620>

Received 24 November 2020; Received in revised form 18 March 2021; Accepted 25 March 2021

Available online 13 April 2021

1567-5769/© 2021 Elsevier B.V. All rights reserved.

1. Introduction

Histamine is a well-known mediator of acute inflammatory and immediate hypersensitivity responses. Mast cells are strategically located in the upper dermis of normal skin, where host tissue is exposed to external antigens and bacteria. Histamine is released from mast cells when tissues are inflamed or stimulated by allergens [1], and once released, histamine-induced itch is triggered by the excitation of a subset of unmyelinated C-fibers [2]. Increased levels of histamine have been described in lesions of eczematous skin diseases and psoriasis and can reach concentrations as high as 10^{-5} – 10^{-3} M during immediate hypersensitivity reactions following mast cell degranulation [3]. In addition to its involvement in immediate-type allergy and dendritic cells maturation, histamine also stimulates human keratinocytes through histamine receptors to increase the expression of anti-microbial peptide, cytokine, chemokine, and matrix metalloproteinase [4,5]. Mast cell activation and histamine release also contribute to skin barrier defects in inflammatory skin diseases [6]. Four types of histamine receptors are known. Antagonists that block H1 or H2 receptors have become successful drug treatments for allergic diseases and gastric acid secretion and H3 receptors have been approved for the treatment of narcolepsy. However, there are no H1, H2, or H3 receptor antagonists that can reduce the inflammation and itching symptoms of chronic inflammatory skin diseases, such as atopic dermatitis (AD) or psoriasis. In the early 2000s, the H4 receptor was described by several groups. Various selective H4 receptor ligands and H4 receptor gene knockout mice can be used to analyze the expression and function of these receptors, thereby revealing the H4 receptor-mediated itching and regulating the relationship between various immune and epithelial cells in cellular responses. In addition, based on initial clinical trials, H4 receptor antagonists can reduce inflammation and scratching behavior in AD patients [7]. Next generation antihistaminic agents possessing H1R and H4R antagonistic actions may be a potent therapeutic drug for AD [8].

Although the TRPV1 channel is known to be located in sensory neurons and the central nervous system, recent evidence suggests that functional TRPV1 is also expressed in human skin and epidermal cells [9]. In 2001, TRPV1 expression on human keratinocytes was first discovered by the immunofluorescence of punch biopsies in primary keratinocytes from healthy volunteers, and TRPV1 mRNA was isolated from primary keratinocytes [10]. TRPV1-mediated calcium influx in cultured human keratinocytes and HaCaT keratinocytes inhibited proliferation and induced apoptosis [11–14]. TRPV1 activation was also associated with the calcium-dependent expression and secretion of cyclooxygenase 2 (COX2), prostaglandin E2, and interleukin 8, indicating that keratinocytes take an active part in skin inflammation [9,15]. TRPV1 channels also seem to be involved in photo-mediated skin aging because of collagen destruction via the induction of matrix metalloproteinase 1 (MMP1) [14]. Lee et al. [14] showed that UV irradiation-mediated calcium influx and the induction of MMP1 was blocked by the TRPV1 blocker capsazepine in HaCaT keratinocytes. Studies using hairless mice confirmed these findings. Some scholars have found a TRPV1-specific blocker inhibited the UV irradiation-induced increase in MMPs as well as proinflammatory cytokines such as interleukin 1 β , 2, 4 and tumor necrosis factor α . It also reduced UV-induced skin thickening [16,17]. These findings prove that the TRPV1 channel on keratinocytes has a similar role to dorsal root ganglion (DRG), and may also play a key role in skin diseases.

Currently, many people are increasingly interested in plant polyphenols as an alternative method for the treatment of inflammatory skin diseases. However, there is a limited understanding of the many mechanisms by which plant phenolic compounds exert anti-inflammatory effects. Nevertheless, the beneficial effects of quercetin (3,3',4',5,7-pentahydroxyflavone) – a flavonoid found in fruits, vegetables, wine, ginkgo, St. John's wort, milk thistle, and other herbs – have been proposed by a number of human nutrition studies. These studies have shown that eating foods rich in quercetin can lead to a reduction in

morbidity and mortality. Quercetin also has anti-inflammatory activity that inhibits cyclooxygenase. In cell culture and animal models, quercetin appears to have anticancer effects. Quercetin is also known for its anti-allergic immune effects. Previous results validated quercetin as an effective small molecule inhibiting Mrgprx2-induced pseudo-allergic reactions via PLC γ -IP3R associated Ca²⁺ fluctuations, thus making it a potential candidate to suppress Mrgprx2 induced pseudo-allergic related diseases [18]. Additionally, quercetin exerted anti-inflammatory effects on the DNCB or MC903-induced AD animal model. Therefore, it may be hypothesized that quercetin treatment may be further developed as a medicine for AD [19,20]. Moreover, quercetin could improve skin inflammation in imiquimod-induced psoriasis mice [21]. Quercetin is also a potential agent against UVB irradiation-induced skin damage [22]. The authors suggest that the protective effect of quercetin against UVB irradiation-induced toxicity is mainly mediated by its ROS scavenging ability. Since histamine causes an increase in intracellular calcium, releasing some substances and causing skin inflammation and photoaging, the purpose of our research is to investigate whether quercetin will affect the mechanics of intracellular calcium increases caused by histamine in keratinocytes. We believe these results will help explain the anti-inflammatory and anti-aging mechanisms of quercetin in the skin.

2. Materials and methods

2.1. Materials

Histamine, quercetin (purity $\geq$ 95%), fexofenadine, ranitidine, ciprofloxacin, JNJ7777120, U73122, GF109203X, capsazepine, and GSK2193874 were purchased from Sigma Chemical Co. (St Louis, MO, USA). 4-methylhistamine (4-MH) was purchased from Tocris Bioscience (Minneapolis, MN, USA).

2.2. Isolation of human primary epidermal keratinocytes

The human primary epidermal keratinocytes were cells isolated from human foreskins. The foreskins were provided by the Mackay Memorial Hospital, after obtaining the consent for use (#18MMHIS039e) from the Institutional Review Board. There was no interaction between the researcher and the foreskin donors, and the foreskins do not have any relevant information to identify the donors. All experiments were performed in accordance with relevant guidelines and regulations. The primary keratinocytes were isolated from human foreskin tissue and were grown in Keratinocyte-SFM (Gibco BRL/Invitrogen, Carlsbad, CA). The primary keratinocytes were used between passages 2 to 4 in this study.

2.3. Spectrofluorometry

[Ca²⁺]_i was measured with fura-2 as described previously [23]. Keratinocytes were resuspended in DMEM containing 5 μ M Fura 2-AM and 1.2 mM CaCl₂ at 37 °C for 30 min. After Fura-2 loading, keratinocytes were pelleted and resuspended in fresh DMEM. An aliquot (2 mL) was transferred to a stirred cuvette containing 1.2 mM CaCl₂. Fura-2-Ca fluorescence was assayed at the excitation wavelengths of 340 and 380 nm (emission wavelength, 505 nm) in a PerkinElmer LS-55 spectrofluorometer (PerkinElmer Life and Analytical Sciences). Data were recorded at 10 s intervals [23].

2.4. Confocal [Ca²⁺]_i image

Keratinocytes were seeded in 96-well cell culture plates (10⁴ cells per well). After cell attaching, a 100 μ L calcium staining solution (FLIPR Calcium 6 assay kit, containing 0.1 mM CaCl₂), was added and the wells were then incubated at 37 °C for two hours. Cells were pretreated with different concentrations of quercetin (3, 10, and 30 μ M) for 20 min

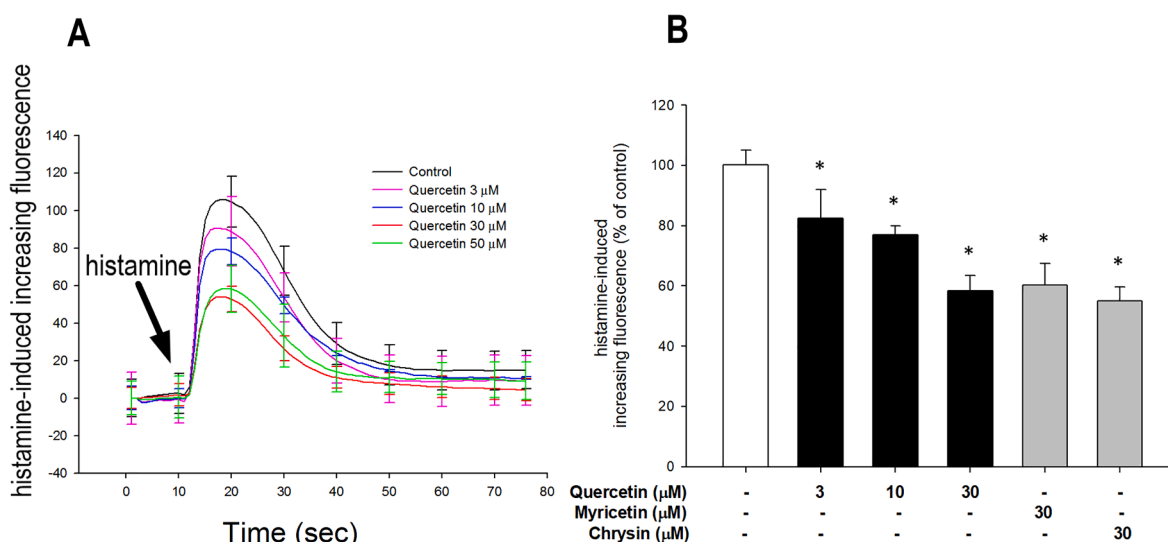


Fig. 1. Histamine induced an increase in the $[Ca^{2+}]_i$ of keratinocyte and quercetin inhibited the $[Ca^{2+}]_i$ elevation. (A) Representative traces of keratinocytes' response to 100 μ M histamine or 20 min pretreatment of quercetin. (B) Histograms show that quercetin exhibited a concentration-dependent decrease in $[Ca^{2+}]_i$ elevation to 100 μ M histamine stimulation. The data represent the means $\pm$ SD. All results are representative of at least three separate experiments. * $P < 0.05$ compared with the control group (Student's t -test).

before the end of the staining. One mM $CaCl_2$ was added before using the ImageXpress Micro Confocal High-Content Imaging System (Molecular Devices). According to the experimental conditions, first, a 10X magnification objective lens was used to shoot the image of the cells before stimulation. Histamine 100 μ M was added to stimulate, then the 30th-second image after stimulation was captured and used to compare the fluorescence performance differences between the groups.

2.5. Real-time quantitative PCR

After four hours of histamine or 4-methylhistamine stimulation with TNF- α , total RNA was isolated using the total RNA isolation kit (GeneDireX®, Vegas, NV) according to the manufacturer's instructions and reverse-transcribed into cDNA using the iScript™ cDNA Synthesis kit (Bio-Rad, Hercules, CA). The qPCR was performed using the StepOne-Plus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, US) with SYBR green (Applied Biosystems, Foster City, CA, US). Primer sequences used in the PCR reactions are IL-8, forward: ACTGAGAGTGATGAGAGTGGAC and reverse: AACCTCTGCACCCAGTTTC and GAPDH, forward: CTGCTCCTCTGTTCGACAGT and reverse: CCGTTGACTCCGACCTTCAC. Data were normalized relative to GAPDH expression and evaluated using the equation: fold change = $2^{-\Delta\Delta CT}$.

2.6. Compound 48/80-induced scratching behavior in BALB/c mice

Quercetin was orally administered to the animals for five days. The rostral part of the skin on the back of mice was clipped, and 20 μ l of compound 48/80 solution was injected intradermally. Control mice received a saline injection instead. Immediately after injection, the animals were put into an observation cage (11 cm in diameter, MicroAct, Neuroscience, Tokyo, Japan), which automatically and objectively detected and evaluated mouse-scratching behavior [24]. Scratching behavior was measured for 60 min. The analysis parameters used by MicroAct to detect waves corresponding to the continuous scratching behavior of mice are as follows: Threshold-0.05 V, Event gap-0.05 s, Minimum duration-0.25 s, Maximum frequency-30 Hz, and Minimum frequency-5 Hz.

2.7. Docking quercetin model

The quercetin molecule was constructed by the following steps: (1)

using GaussView 6.0 software to build the initial geometry; (2) employing density functional theoretical (DFT) calculations to obtain the optimized structure at the B3LYP/6-31 g* level [25] (the solvent effects of DMSO were included using the polarizable continuum model (PCM) [26]); (3) performing the frequency calculation to confirm the output structure displays the equilibrium geometry; and (4) converting the optimal quercetin file into PDBQT format for the docking study. Steps 2 and 3 were executed by means of the Gaussian 16 package, while step 4 was succeeded in AutodockTools-1.5.6 [27]. The protein sequence of the mouse H4 receptor was obtained from the UniProt database (<http://www.uniprot.org>; UniProtKB Entry: Q9H3N8). After that, the structure of H4 was constructed through the templated-based protein structure modeling method with the Phyre2 server [28]. The results showed that the human muscarinic receptor M4 (pdbid: 6KP6) is the best template. The PDBQT format of the modeled three-dimension protein structure was then obtained after adding hydrogens using the AutodockTools-1.5.6 package. The theoretical binding energy between quercetin and H4 receptors was calculated via Autodock Vina [29]. The cubical grid for atomic interaction energy was an $80 \times 66 \times 104$ box with 1.00 Å spacing centered on a protein binding pocket. The resultant docking structure was then displayed by Discovery Studio Client v19.1.0.18287 software.

2.8. Statistical analysis

Data were expressed as the mean $\pm$ SD by using GraphPad Prism Program 6 software (GraphPad Software San Diego, CA). Student's t -test for comparisons were used to compare the mean difference between groups with and without quercetin, inhibitors, or blockers treatment. We considered $p < 0.05$ to be statistically significant.

3. Results

3.1. Inhibitory effect of quercetin on histamine induced $[Ca^{2+}]_i$ elevation

As shown in Fig. 1, histamine (100 μ M) induced an obvious increase of $[Ca^{2+}]_i$ concentration in keratinocytes. The $[Ca^{2+}]_i$ response consisted of an initial rise, a slow decay, and a sustained phase. Furthermore, after pretreatment with quercetin, the $[Ca^{2+}]_i$ concentration was significantly inhibited (Fig. 1A). The inhibitory effect of quercetin is dose-dependent at concentrations below 30 μ M. The IC_{50} is about 16.0

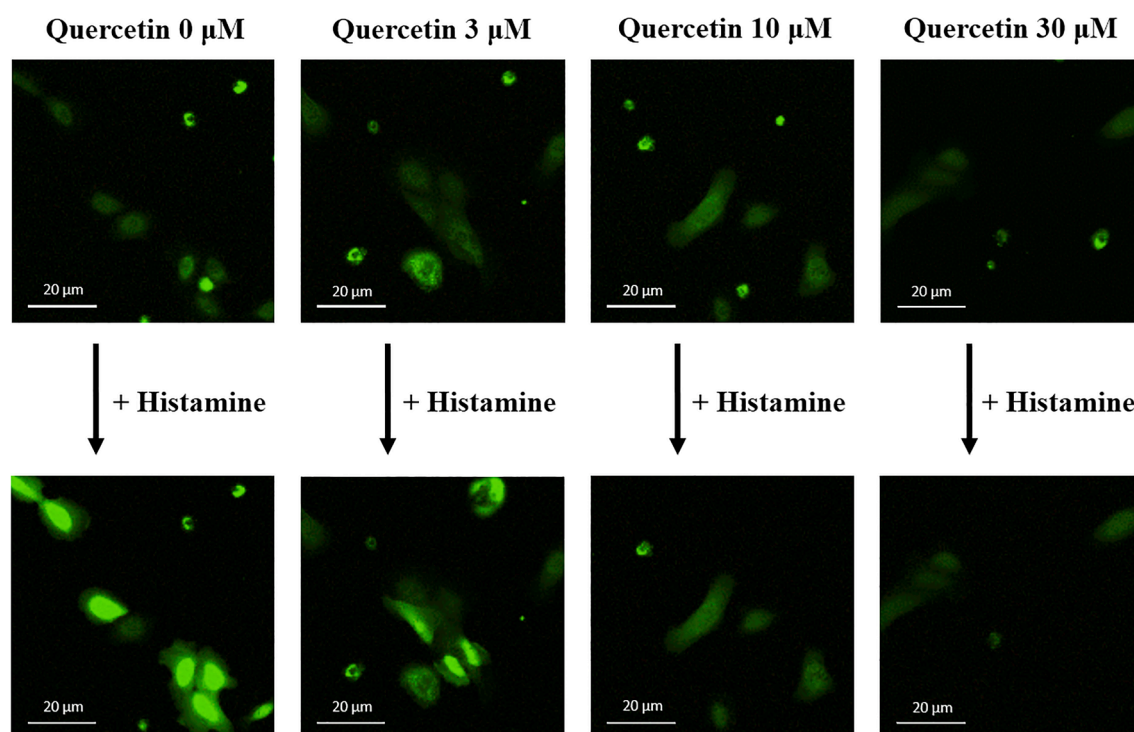


Fig. 2. The image of quercetin inhibited the $[Ca^{2+}]_i$ rise using a confocal laser-scanning microscope. The fluorescence intensity of FLIPR Calcium 6 assay kit loaded keratinocyte in response to histamine was recorded in the presence or absence of quercetin (3, 10, and 30 μ M), the images of intracellular Ca^{2+} changes in representative cells.

μ M (Fig. 1B). Furthermore, we tested whether myricetin, another flavonol compound, and chrysin, a flavon compound, also have the same effects. As shown in Fig. 1B, we found that these plant polyphenol compounds all have the same inhibitory effect. In order to more clearly see the inhibitory effect of quercetin, we further applied a confocal microscope to observe the change of calcium ions. As shown in Fig. 2, the intensity of the fluorescence weakened as the dose of quercetin increased. This confirmed that quercetin can inhibit $[Ca^{2+}]_i$ elevation caused by histamine.

3.2. Inhibitory effect of quercetin is blocked by a H4 receptor blocker but not H1, H2, and H3 blockers

A previous study revealed that the application of histamine receptor antagonists could inhibit the histamine-induced $[Ca^{2+}]_i$ elevation in cultured normal human epidermal keratinocytes [30]. Therefore, we verified whether quercetin also inhibits histamine-induced $[Ca^{2+}]_i$ elevation via histamine receptors. As shown in Fig. 3A and B, the selective histamine receptor antagonists inhibited histamine-induced $[Ca^{2+}]_i$ elevation to different degrees. Quercetin still inhibited histamine-induced $[Ca^{2+}]_i$ elevation in the presence of H1, H2, and H3 selective blockers. However, quercetin failed to produce any inhibition in the presence of the H4 receptor blocker (JNJ777120). These results suggest that the inhibitory effect of quercetin on histamine-induced $[Ca^{2+}]_i$ elevation is mediated by H4 receptor. In addition, we investigated whether the inhibitory effect of quercetin in the presence of different concentrations of H4 receptor blockers can be blocked in a concentration-related manner. As shown in Fig. 3B, we found that the H4 blocker could increase its blocking effect as its concentration increases. Indeed, it could completely block the inhibitory effect of quercetin after treatment of 10 μ M. In order to prove the inhibitory effect of quercetin via H4 receptors, we used selective H4 receptor agonists to increase intracellular calcium concentration. As shown in Fig. 3C, the increase in intracellular calcium induced by H4 can be completely inhibited by H4 receptor blockers and quercetin, respectively.

3.3. Inhibitory effect of quercetin also be blocked by PLC and PKC pathway inhibitor

Activated H4 receptor, Immeipip, -induced scratching behavior could be blocked by phospholipase C (PLC) pathway inhibitor U73122. PLC plays a key role in the signaling pathway links of GPCRs to an intracellular signaling network. Activated H4 receptors stimulate PLC, with the subsequent generation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). These two mediators are known to elevate $[Ca^{2+}]_i$ and to activate PKC, respectively [31]. Both intracellular and extracellular Ca^{2+} sources are required to initiate and maintain responses. As shown in Fig. 4A, quercetin did not further inhibit histamine-induced $[Ca^{2+}]_i$ elevation after pretreatment of U73122, a PLC inhibitor. Moreover, we also found that quercetin did not further inhibit the increase of intracellular calcium ions caused by histamine in the presence of the PKC inhibitor GF109203X (Fig. 4B).

3.4. Selective TRPV1 blocker inhibits the effect of quercetin on histamine induced $[Ca^{2+}]_i$ elevation

As shown in Fig. 4C, we found that quercetin decreased histamine-induced $[Ca^{2+}]_i$ elevation to approximately 62.7%. The TRPV1 antagonist, capsazepine, decreased the response to histamine to approximately 53.5%. However, we found that quercetin did not further inhibit $[Ca^{2+}]_i$ elevation caused by histamine in the presence of capsazepine (Fig. 4C). Furthermore, we conducted a similar experiment. After pretreatment with the TRPV4 antagonist, GSK2193874, quercetin can still inhibit $[Ca^{2+}]_i$ elevation caused by histamine (Fig. 4D).

3.5. Quercetin could inhibit histamine-induced IL-8 mRNA expression

IL-8 mRNA expression might be enhanced by histamine and TNF α via H4 receptor stimulation in keratinocytes [32]. Therefore, we further verify that quercetin can inhibit IL-8 mRNA expression induced by histamine. Fig. 5 shows that quercetin and capsazepine can inhibit the IL-8

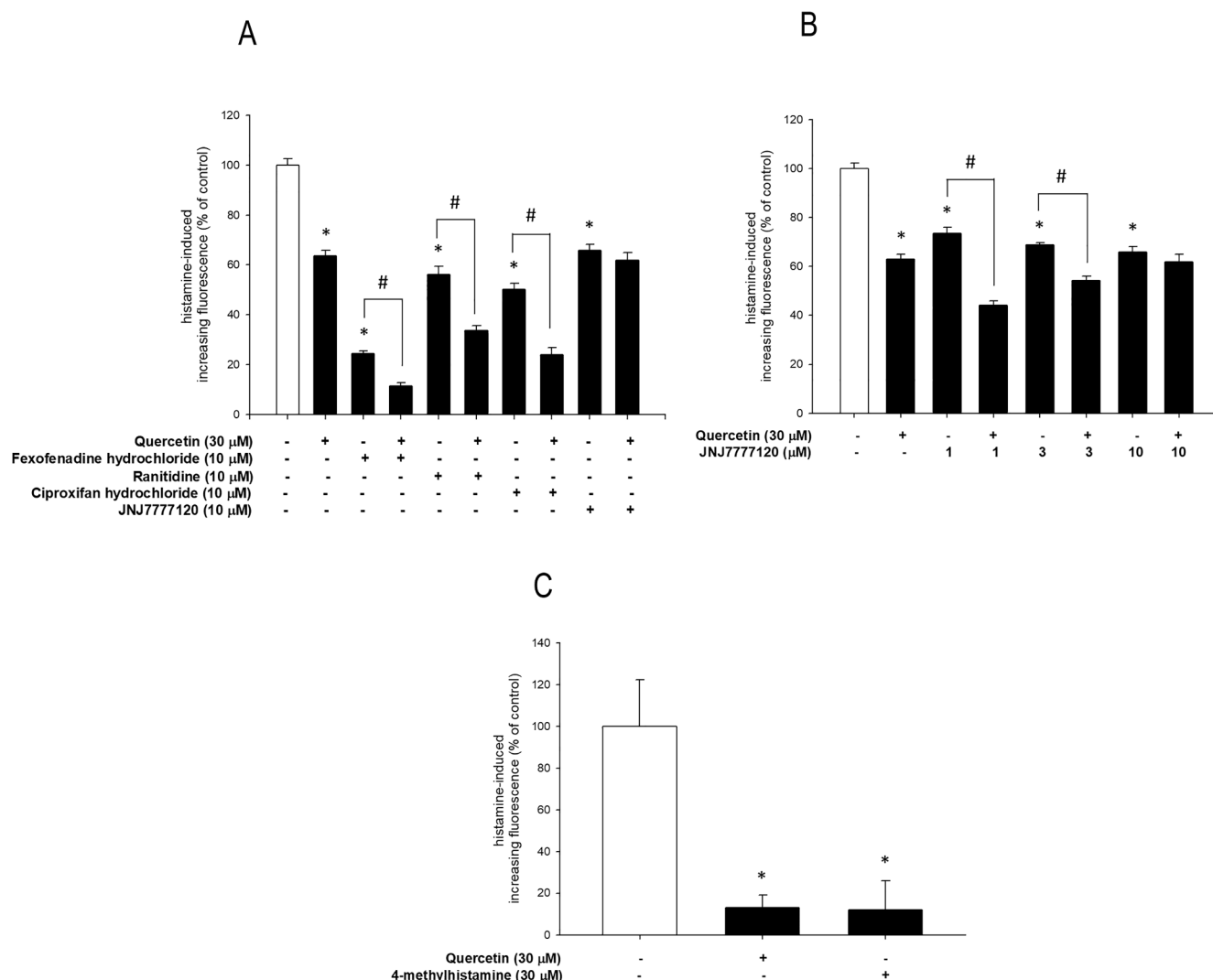


Fig. 3. The $[Ca^{2+}]_i$ elevation-induced by histamine or 4MH (H4 receptor agonist) was affected by quercetin and histamine receptor blockers (H1: fexofenadine; H2 blocker: ranitidine; H3 blocker: ciproxifan; H4 blocker: JNJ777120). (A) The effect of quercetin on $[Ca^{2+}]_i$ elevation caused by histamine in the presence of H1, H2, H3, and H4 receptor blockers. In the presence of H4 receptor blockers, quercetin is unable to produce the inhibitory effect of histamine-induced $[Ca^{2+}]_i$ elevation. (B) The inhibitory effect of quercetin in the presence of different concentrations of H4 receptor blocker. (C) The $[Ca^{2+}]_i$ elevation-induced by 4MH was inhibited by H4 receptor blocker and quercetin. The data represent the means $\pm$ SD. All results are representative of at least three separate experiments. *, # $P < 0.05$ compared with the control group or blocker alone (Student's *t*-test).

mRNA expression by co-treatment of TNF α and histamine. Moreover, we also find that quercetin inhibits the IL-8 mRNA expression by co-treatment of TNF α and 4-methylhistamine.

3.6. Quercetin could inhibit compound 48/80-induced scratching behavior in BALB/c mice

We further proved that quercetin has an anti-histamine-induced scratching effect. We used a histamine-releasing agent, compound 48/80, to induce scratching behavior in BALB/c mice. This animal model proved that quercetin has an anti-histamine-induced scratching effect. As shown in Fig. 6, we found that the scratching behavior induced by compound 48/80 (50 μ g/site) was inhibited by the treatment of quercetin. Repeated administration of lower and higher doses of quercetin (3 and 10 mg/kg) for 5 days greatly reduced the number of scratches caused by compound 48/80.

3.7. The docking interaction of quercetin with H4 receptors

Finally, a molecular docking method was used to predict whether

there exists a direct interaction between quercetin and H4 receptors. As shown in Fig. 6, the molecular docking study shows that quercetin could bind well with H4 receptors with a binding affinity of -8.7 kcal/mol. The docking complex 2D and 3D structures reveal that the ligand-receptor interactions were mainly contributed by a π - π T-shaped with a B and C ring of amino acid Tyr 72 and Phe 344. On the other hand, the ligand-receptor interactions exhibit π -alkyl interactions of amino acid Pro 166, Leu 91, and Lys164 with quercetin. The predicted structure shows an unfavorable acceptor-acceptor interaction of Leu71 with the oxygen atom from B ring in quercetin and also exhibits van der Waals interactions of amino acid with quercetin (Fig. 7A and B).

4. Discussion

In this study, we found that TRPV1, PLC, and PKC, but not TRPV4, are involved in the inhibition of histamine-induced calcium influx in keratinocytes via H4 receptors activation by quercetin. We found that this effect of quercetin may contribute to its anti-allergic and inflammatory skin diseases and anti-photoaging effects.

UV light, especially UVB, is thought to be the cause of allergic

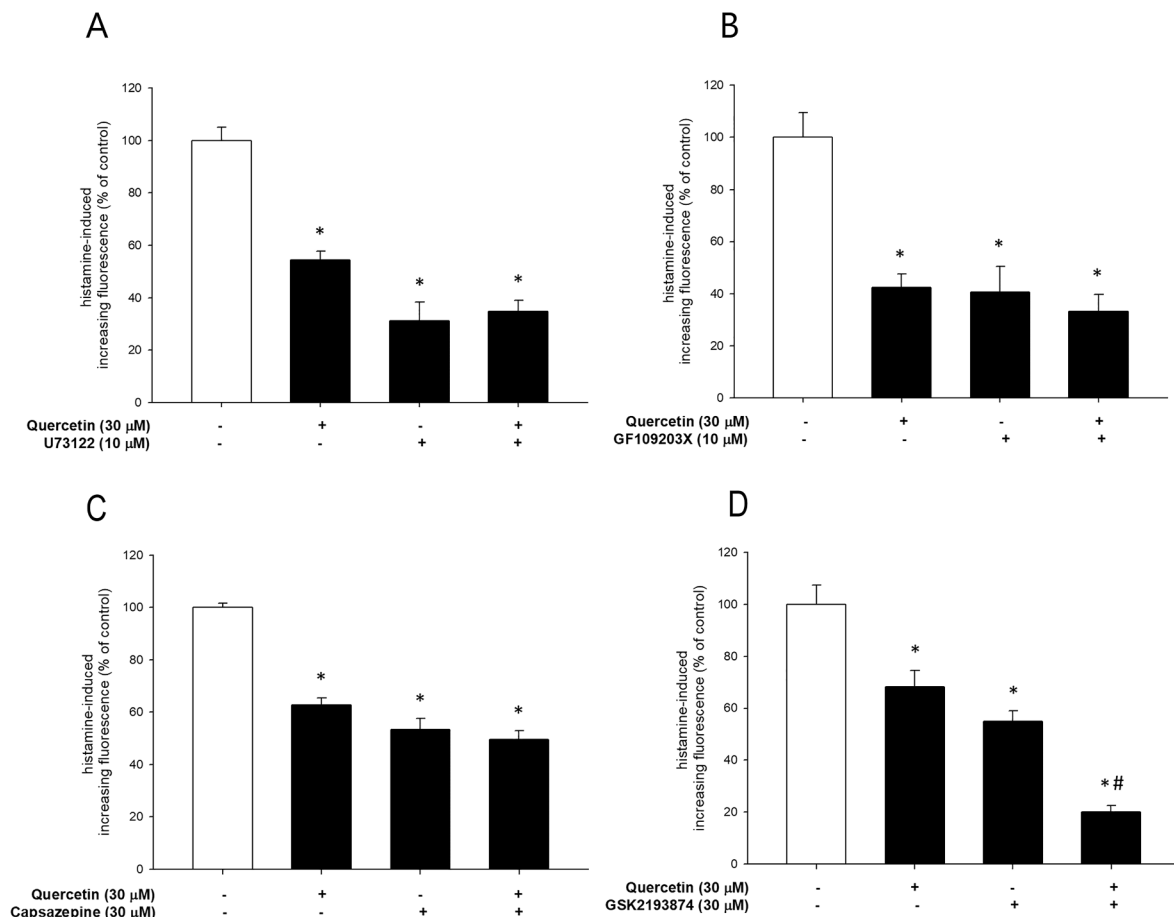


Fig. 4. The effect of quercetin on $[Ca^{2+}]_i$ elevation caused by histamine in the presence of: (A) PLC inhibitors (U73122), (B) PKC inhibitors (GF109203X), (C) a selective TRPV1 blocker (capsazepine), and (D) TRPV4 blockers (GSK2193874). In the presence of PLC inhibitors, PKC inhibitors, and TRPV1 blockers, quercetin cannot produce the inhibitory effect of histamine-induced $[Ca^{2+}]_i$ elevation. However, in the presence of TRPV4 blockers, quercetin still has the inhibitory effect of histamine-induced $[Ca^{2+}]_i$ elevation. The data represent the means $\pm$ SD. All results are representative of at least three separate experiments. * $P < 0.05$ compared with the control group and # $P < 0.05$ compared with GSK2193874 alone group (Student's *t*-test).

reactions and could cause mast cell degranulation and histamine release. Notably, it has been reported that mast cell activation is involved in the UV-induced sunburn reaction [33]. Therefore, our research results show that quercetin inhibits the increase of intracellular calcium caused by histamine and may have an effect against ultraviolet B damage. The UV-induced MMP-1 expression in HaCaT was also decreased by TRPV1 inhibitors and was facilitated by capsaicin. Knock-down of TRPV1 using siRNA transfection also decreased MMP-1 expression, as well as UV-induced Ca^{2+} influx in HaCaT [14]. Our results also show the presence of TRPV1 inhibitors could block the inhibitory effects of quercetin. This result may contribute to the effect of quercetin against ultraviolet B and sunburn. Previous results also suggest that the protective effect of quercetin against UVB irradiation-induced toxicity is mainly mediated by the ROS scavenging ability [22]. Quercetin might reduce the production of MMP-1 by reducing histamine released by UVB radiation. This would decrease intracellular calcium and thereby reduce MMP-1's ability to produce damage. However, this is different from quercetin's antioxidant effect against UVB. We will study this further in the future.

Mast cells are strategically located in the upper dermis of normal skin, where host tissue is exposed to external antigens and bacteria.

After activation by a range of stimuli, mast cells release histamine [11]. Histamine is involved in allergic and inflammatory reactions [34] by binding to one of the four known G-protein coupled transmembrane H1-H4 receptors. These receptors are expressed on various cell types including monocytes, lymphocytes, dendritic cells, and keratinocytes [35]. Histamine is recognized when binding histamine receptors activate the histamine signaling pathway and trigger important intracellular signaling pathways, such as PLC and adenylate cyclase pathways. This causes intracellular calcium increases from calcium influx and calcium release, leading to the production of various proinflammatory cytokines. Previous studies also showed that the enhanced intracellular Ca^{2+} levels appear to account for different pharmacological properties promoted through the receptor [36]. Among them, there have been extensive studies in recent years about the H4 receptor's function in histamine-dependent itch in animal behavior models [2,6]. The results further indicate that TRPV1 is an important ion channel in the induction of DRG neurons' responses in the downstream signaling pathway of histamine H4 receptors. This suggests that TRPV1 may be involved in the mechanism of histamine-induced itch response by H4 receptor activation [37]. However, the ionic mechanism and downstream signal pathway of

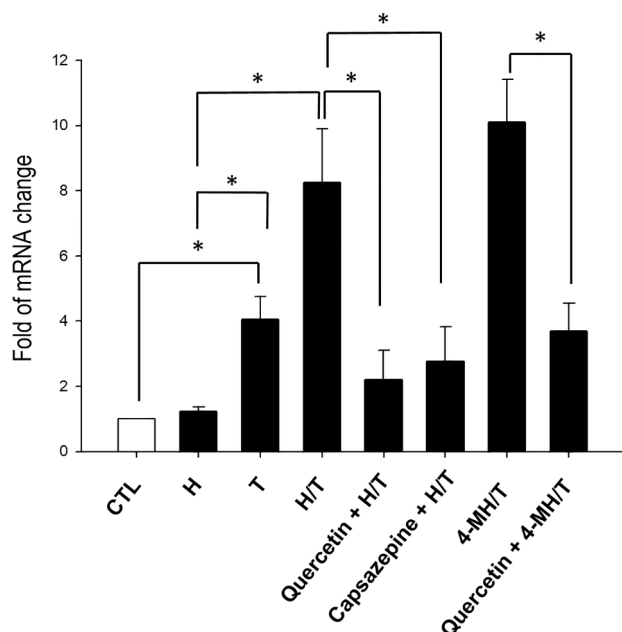


Fig. 5. The effects of quercetin and capsazepine on the expression of IL-8 mRNA by histamine- or 4-methylhistamine and TNF α -treated keratinocytes. Cells were cultured with histamine, 4-MH, TNF α and quercetin as indicated. mRNA levels were normalized to the corresponding GAPDH mRNA levels, and the mean value in the control group was set to 1.0. The data represent the means $\pm$ SD. All results are representative of at least three separate experiments. * $P < 0.05$ signifies the comparison between the two groups is significant (Student's *t*-test). H: histamine; T: TNF α ; 4-MH: 4-methylhistamine.

activation of H4 receptors on keratinocyte are still unclear. In the present study, we have revealed that TRPV1, PLC, and PKC could be involved in the histamine-induced calcium influx on keratinocyte by activation of H4 receptors.

Pruritogens, including amines, proteases, and cytokines, have been

implicated in the induction of itch. These include thymic stromal lymphopoietic protein (TSLP) [38], histamine [39], 5-HT [5], Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL) [40], substance P [41], and IL-31 [42]. Moreover, some studies have showed that stimulation of H4 receptors up-regulates the production of IL-31 in Th2 cells and TSLP release from keratinocytes [7]. Furthermore, IL-8 mRNA expression might be enhanced by histamine and TNF α via H4 receptor stimulation in keratinocytes [32]. Therefore, H4 receptors are expressed on keratinocytes, which play a role in the inflamed skin of AD. Moreover, in initial clinical trials, H4 receptor antagonists reduced inflammation and scratching behavior in patients with AD [7]. The H4 receptor antagonist toreforant (JNJ-38518168) has completed efficacy studies in psoriasis [7]. A study also demonstrated that topical administration of quercetin plays a beneficial role in controlling AD symptoms and thus may serve as potential candidate for AD treatment [20]. In addition, quercetin could ameliorate imiquimod-induced psoriasis-like skin inflammation in mice [21]. Based on the present data, quercetin inhibits the scratching behavior-induced compound 48/80. Therefore, our results indicate that the inhibition of H4 receptor function by quercetin may also help it alleviate allergic inflammatory skin diseases. In future research, more direct evidence will be confirmed. On the other hand, our results show that quercetin, myricetin, and chrysin have similar inhibitory effects. Their structures belong to the flavonoids. However, we have found that isoflavonoids only slightly inhibit the effect of histamine-induced $[Ca^{2+}]_i$ elevation (data not shown). Potential causes for this phenomenon include the following: (1) the different position of the B ring in the structure and the different bind affinity to the histamine receptor; (2) the electrical resonance of flavonoids structure is better than isoflavonoids. Therefore, we suggest that flavonoid structures may have a better anti-itching effect on histamine than isoflavonoids, but further studies need to confirm this hypothesis.

5. Conclusions

These data, together with past literature data, show that anti-histamine induced calcium influx via TRPV1 may provide a molecular mechanism of quercetin in anti-itching and unpleasant sensations. Quercetin could affect the TRPV1 channel through H4 receptors and PLC and PKC signaling pathways in keratinocytes to reduce intracellular

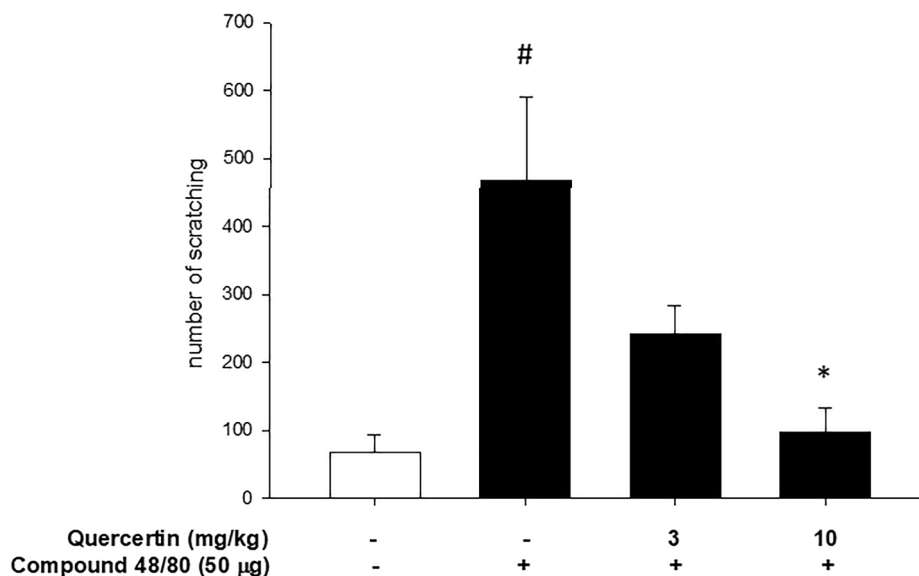


Fig. 6. The Effects of quercetin for treatment of 5 days on Compound 48/80 -Induced Scratching Behavior in BALB/c mice. Each column and vertical bar show the mean $\pm$ SD ($n = 3$). #, * $P < 0.05$ compared with the control group and compound 48/80 alone group (Student's *t*-test).

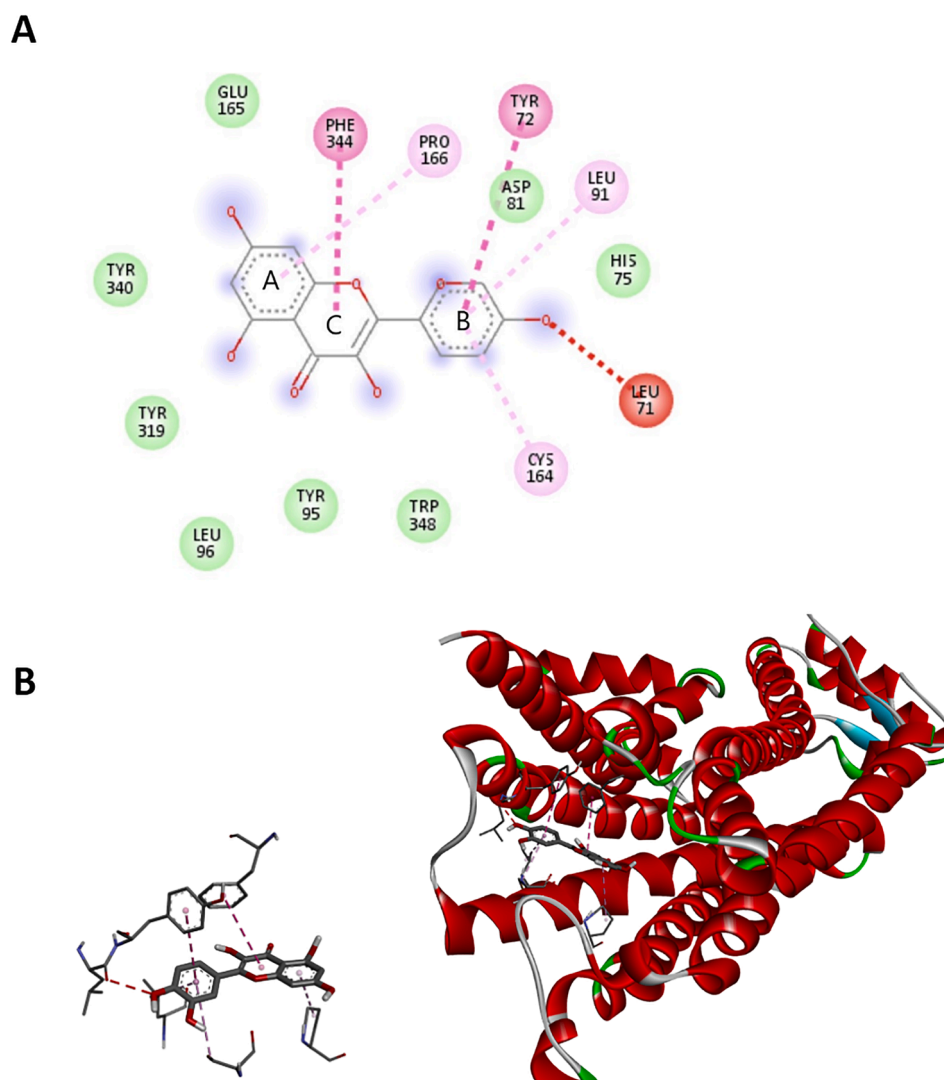


Fig. 7. Quercetin docked with H4 receptors. (A) 2D structure of the docking interaction between quercetin and H4 receptors. Color code for interactions: green- van der Waals; deep pink- Pi-Pi T-shaped; light pink- Pi-Alkyl; red- unfavorable acceptor. (B) 3D structure of predicted complex and the docking interaction of quercetin with H4 receptors.

calcium concentration, and has shown therapeutic potential as an anti-inflammatory and anti-allergic drug in the skin.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is supported by research grants from Fu-Jen Catholic University, Ministry of Science and Technology (MOST107-2320-B-030-003-MY3), Taoyuan Armed Forces General Hospital (AFTYGH-10808), and the Cathay General Hospital (105-CGH-FJU-05) in Taiwan.

References

- [1] E. Tiligada, Editorial: Is histamine the missing link in chronic inflammation? *J. Leukoc. Biol.* 92 (1) (2012) 4–6.
- [2] W.-S. Shim, U. Oh, Histamine-Induced Itch and its Relationship with Pain, *Molecular Pain* 4 (2008) 29.
- [3] R. Gutzmer, M. Gschwandtner, K. Rossbach, S. Mommert, T. Werfel, M. Kietzmann, W. Baeumer, Pathogenetic and therapeutic implications of the histamine H4 receptor in inflammatory skin diseases and pruritus, *Front Biosci. (Schol. Ed.)* 3 (2011) 985–994.
- [4] M.L. Giustizieri, C. Albanesi, J. Fluhr, P. Gisondi, J. Norgauer, G. Girolomoni, H1 histamine receptor mediates inflammatory responses in human keratinocytes, *J. Allergy Clin. Immunol.* 114 (5) (2004) 1176–1182.
- [5] N. Kanda, S. Watanabe, Histamine enhances the production of human beta-defensin-2 in human keratinocytes, *Am. J. Physiol. Cell Physiol.* 293 (6) (2007) C1916–C1923.
- [6] M. Gschwandtner, M. Mildner, V. Mlitz, F. Gruber, L. Eckhart, T. Werfel, R. Gutzmer, P.M. Elias, E. Tschachler, Histamine suppresses epidermal keratinocyte differentiation and impairs skin barrier function in a human skin model, *Allergy* 68 (1) (2013) 37–47.
- [7] K. Schaper-Gerhardt, K. Rossbach, E. Nikolouli, T. Werfel, R. Gutzmer, S. Mommert, The role of the histamine H 4 receptor in atopic dermatitis and psoriasis, *Br. J. Pharmacol.* 177 (3) (2020) 490–502.
- [8] Y. Ohsawa, N. Hirasawa, The role of histamine H1 and H4 receptors in atopic dermatitis: from basic research to clinical study, *Allergol. Int.* 63 (4) (2014) 533–542.
- [9] M.D. Southall, T. Li, L.S. Gharibova, Y. Pei, G.D. Nicol, J.B. Travers, Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes, *J. Pharmacol. Exp. Ther.* 304 (1) (2003) 217–222.
- [10] M. Denda, S. Fuziwara, K. Inoue, S. Denda, H. Akamatsu, A. Tomitaka, K. Matsunaga, Immunoreactivity of VR1 on epidermal keratinocyte of human skin, *Biochem. Biophys. Res. Commun.* 285 (5) (2001) 1250–1252.
- [11] C. Radtke, N. Sinis, M. Sauter, S. Jahn, U. Kraushaar, E. Guenther, H.P. Rodemann, H.O. Rennekampff, TRPV channel expression in human skin and possible role in thermally induced cell death, *J. Burn Care Res.* 32 (1) (2011) 150–159.
- [12] E. Bodo, T. Biro, A. Telek, G. Czifra, Z. Griger, B.I. Toth, A. Mescalcin, T. Ito, A. Bettermann, L. Kovacs, R. Paus, A hot new twist to hair biology: involvement of

- vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control, *Am. J. Pathol.* 166 (4) (2005) 985–998.
- [13] B.I. Toth, N. Dobrosi, A. Dajnoki, G. Czifra, A. Olah, A.G. Szollosi, I. Juhasz, K. Sugawara, R. Paus, T. Biro, Endocannabinoids modulate human epidermal keratinocyte proliferation and survival via the sequential engagement of cannabinoid receptor-1 and transient receptor potential vanilloid-1, *J. Invest. Dermatol.* 131 (5) (2011) 1095–1104.
- [14] Y.M. Lee, Y.K. Kim, K.H. Kim, S.J. Park, S.J. Kim, J.H. Chung, A novel role for the TRPV1 channel in UV-induced matrix metalloproteinase (MMP)-1 expression in HaCaT cells, *J. Cell. Physiol.* 219 (3) (2009) 766–775.
- [15] J. Huang, L. Ding, D. Shi, J.H. Hu, Q.G. Zhu, S. Gao, L. Qiu, Transient receptor potential vanilloid-1 participates in the inhibitory effect of ginsenoside Rg1 on capsaicin-induced interleukin-8 and prostaglandin E2 production in HaCaT cells, *J. Pharm. Pharmacol.* 64 (2) (2012) 252–258.
- [16] F. Elsholz, C. Harteneck, W. Muller, K. Friedland, Calcium—a central regulator of keratinocyte differentiation in health and disease, *Eur. J. Dermatol.* 24 (6) (2014) 650–661.
- [17] Y.M. Lee, S.M. Kang, S.R. Lee, K.H. Kong, J.Y. Lee, E.J. Kim, J.H. Chung, Inhibitory effects of TRPV1 blocker on UV-induced responses in the hairless mice, *Arch. Dermatol. Res.* 303 (10) (2011) 727–736.
- [18] Lee Shin, Hong, Lim, Byun, Quercetin Directly Targets JAK2 and PKC δ and Prevents UV-Induced Photoaging in Human Skin, *Int. J. Mol. Sci.* 20 (21) (2019) 5262.
- [19] H.N. Lee, S.A. Shin, G.S. Choo, H.J. Kim, Y.S. Park, B.S. Kim, S.K. Kim, S.D. Cho, J. S. Nam, C.S. Choi, J.H. Che, B.K. Park, J.Y. Jung, Anti-inflammatory effect of quercetin and galangin in LPS-stimulated RAW264.7 macrophages and DNCB-induced atopic dermatitis animal models, *Int. J. Mol. Med.* 41 (2017) 888–898.
- [20] D.D. Hou, W. Zhang, Y.L. Gao, Y.Z. Sun, H.X. Wang, R.Q. Qi, H.D. Chen, X.H. Gao, Anti-inflammatory effects of quercetin in a mouse model of MC903-induced atopic dermatitis, *Int. Immunopharmacol.* 74 (2019) 105676.
- [21] H. Chen, C. Lu, H. Liu, M. Wang, H. Zhao, Y. Yan, L. Han, Quercetin ameliorates imiquimod-induced psoriasis-like skin inflammation in mice via the NF- κ B pathway, *Int. Immunopharmacol.* 48 (2017) 110–117.
- [22] X. Zhu, N. Li, Y. Wang, L. Ding, H. Chen, Y. Yu, X. Shi, Protective effects of quercetin on UVB irradiation-induced cytotoxicity through ROS clearance in keratinocyte cells, *Oncol. Rep.* 37 (1) (2017) 209–218.
- [23] I.C. Su, C.F. Hung, C.N. Lin, S.K. Huang, S.J. Wang, Cycloheterophyllin inhibits the Release of Glutamate from Nerve Terminals of the Rat Hippocampus, *Chem. Res. Toxicol.* 32 (8) (2019) 1591–1598.
- [24] N. Inagaki, K. Igeta, J.F. Kim, M. Nagao, N. Shiraishi, N. Nakamura, H. Nagai, Involvement of unique mechanisms in the induction of scratching behavior in BALB/c mice by compound 48/80, *Eur. J. Pharmacol.* 448 (2–3) (2002) 175–183.
- [25] P.J. Stephens, F.J. Devlin, C.F. Chabalowski, M.J. Frisch, Ab Initio Calculation of Vibrational Absorption and Circular Dichroism Spectra Using Density Functional Force Fields, *J. Phys. Chem.* 98 (45) (1994) 11623–11627.
- [26] J. Tomasi, B. Mennucci, R. Cammi, Quantum Mechanical Continuum Solvation Models, *Chem. Rev.* 105 (8) (2005) 2999–3094.
- [27] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A. J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *J. Comput. Chem.* 30 (16) (2009) 2785–2791.
- [28] L.A. Kelley, S. Mezulis, C.M. Yates, M.N. Wass, M.J. Sternberg, The Phyre2 web portal for protein modeling, prediction and analysis, *Nat. Protoc.* 10 (6) (2015) 845–858.
- [29] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *J. Comput. Chem.* 31 (2) (2010) 455–461.
- [30] H. Koizumi, H2 histamine receptor-mediated increase in intracellular Ca $^{2+}$ in cultured human keratinocytes, *J. Dermatol. Sci.* 21 (2) (1999) 127–132.
- [31] C. Hisatsune, K. Nakamura, Y. Kuroda, T. Nakamura, K. Mikoshiba, Amplification of Ca $^{2+}$ signaling by diacylglycerol-mediated inositol 1,4,5-trisphosphate production, *J. Biol. Chem.* 280 (12) (2005) 11723–11730.
- [32] E. Suwa, K. Yamaura, S. Sato, K. Ueno, Increased expression of the histamine H4 receptor following differentiation and mediation of the H4 receptor on interleukin-8 mRNA expression in HaCaT keratinocytes, *Exp. Dermatol.* 23 (2) (2014) 138–140.
- [33] B.A. Gilchrest, N.A. Soter, J.S. Stoff, M.C. Mihm Jr., The human sunburn reaction: histologic and biochemical studies, *J. Am. Acad. Dermatol.* 5 (4) (1981) 411–422.
- [34] H. Shirasaka, E. Kanaizumi, N. Seki, T. Himi, Localization and upregulation of the nasal histamine H1 receptor in perennial allergic rhinitis, *Mediators Inflamm.* 2012 (2012) 951316.
- [35] U. Wolfle, B. Haarhaus, C.M. Schempp, Amarogentin Displays Immunomodulatory Effects in Human Mast Cells and Keratinocytes, *Mediators Inflamm.* 2015 (2015) 630128.
- [36] I.T. Harvima, Induction of matrix metalloproteinase-9 in keratinocytes by histamine, *J. Invest. Dermatol.* 128 (12) (2008) 2748–2750.
- [37] T. Jian, N. Yang, Y. Yang, C. Zhu, X. Yuan, G. Yu, C. Wang, Z. Wang, H. Shi, M. Tang, Q. He, L. Lan, G. Wu, Z. Tang, TRPV1 and PLC Participate in Histamine H4 Receptor-Induced Itch, *Neural Plast* 2016 (2016) 1682972.
- [38] T.P. Moran, B.P. Vickery, The Epithelial Cell-Derived Atopic Dermatitis Cytokine TSLP Activates Neurons to Induce Itch, *Pediatrics* 134 (Supplement) (2014) S160–S161.
- [39] W.S. Shim, M.H. Tak, M.H. Lee, M. Kim, M. Kim, J.Y. Koo, C.H. Lee, M. Kim, U. Oh, TRPV1 Mediates Histamine-Induced Itching via the Activation of Phospholipase A2 and 12-Lipoxygenase, *J. Neurosci.* 27 (9) (2007) 2331–2337.
- [40] S.G. Shimada, K.A. Shimada, J.G. Collins, Scratching behavior in mice induced by the proteinase-activated receptor-2 agonist, SLIGRL-NH2, *Eur. J. Pharmacol.* 530 (3) (2006) 281–283.
- [41] T. Andoh, T. Nagasawa, M. Satoh, Y. Kuraishi, Substance P induction of itch-associated response mediated by cutaneous NK1 tachykinin receptors in mice, *J. Pharmacol. Exp. Ther.* 286 (3) (1998) 1140–1145.
- [42] F. Cevikbas, X. Wang, T. Akiyama, C. Kempkes, T. Savinko, A. Antal, G. Kukova, T. Buhl, A. Ikoma, J. Buddenkotte, V. Soumelis, M. Feld, H. Alenius, S.R. Dillon, E. Carstens, B. Homey, A. Basbaum, M. Steinhoff, A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1, *J. Allergy Clin. Immunol.* 133 (2) (2014) 448–460.e7.