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Original Research Article

Bergenin attenuates TNF-α-induced oxidative stress and inflammation in HaCaT cells by activating Nrf2 pathway and inhibiting NF-κB pathway

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Abstract

Purpose: *To investigate the effect of bergenin in TNF-α-induced human immortalized keratinocytes.*
Methods: *Human immortal keratinocyte cells (HaCaT) were incubated with tumor necrosis factor-α (TNF-α), and then treated with increasing concentrations of bergenin. Cell viability was determined using Cell Counting Kit-8 (CCK8), while oxidative stress and inflammation were evaluated using enzyme linked immunosorbent assay (ELISA).*
Results: *Incubation with increasing concentrations of bergenin showed no significant effect on cell viability of HaCaT ($p < 0.05$). Tumor necrosis factor-α significantly induced up-regulation of interleukin (IL)-6 and IL-8 in HaCaT ($p < 0.001$), while bergenin significantly reduced the levels of IL-6 and IL-8 ($p < 0.001$). Bergenin also attenuated TNF-α-induced decrease in superoxide dismutase (SOD), as well as increase in malondialdehyde (MDA) and inducible nitric oxide synthase (iNOS). Protein expression of IκBα in HaCaT was decreased, while p-p65 and p-IκBα were increased by treatment with TNF-α. However, bergenin increased IκBα but decreased p-p65 and p-IκBα in TNF-α-induced HaCaT. Moreover, bergenin reduced Kelch-like ECH-associated protein 1 (Keap1), while enhancing transcription factor NF-E2 p45-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) in TNF-α-induced HaCaT.*
Conclusion: *Bergenin exerts antioxidant and anti-inflammatory effects on TNF-α-induced HaCaT by activating Nrf2 pathway and inactivating NF-κB pathway. Therefore, bergenin is a potential agent for the treatment of psoriasis.*

Keywords: *Bergenin, Oxidative stress, Inflammation, tumor necrosis factor-α, human immortalized keratinocytes, NF-E2 p45-related factor 2, psoriasis*

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INTRODUCTION

Psoriasis, a recurrent inflammatory skin disease, is difficult to treat [1] It is generally accompanied with comorbidities, such as tonsillitis, cardiovascular diseases, diabetes mellitus and hypertension [2]. Therefore, novel strategies are needed for the prevention of psoriasis. A previous study has shown that psoriatic plaques are characterized by increased dermal capillary

density and permeability, dermal infiltration of immune cells, and aberrant proliferation and differentiation of keratinocytes [3]. Suppression of hyperproliferation in keratinocytes has been regarded as an effective strategy for the treatment of psoriasis [4]. Oxidative stress has been found to be implicated in the pathogenesis of psoriasis, through the promotion of inflammatory cytokines and growth factors, contributing to sustained skin inflammation and keratinocyte over-proliferation [5]. Therefore, anti-oxidant and anti-inflammatory strategies show promising effect against psoriasis [6].

Bergenin, an isocoumarin isolated from *Saxifraga stolonifera* Curt., has been widely used in neuroprotection and liver protection, and it exerts anti-tumor, anti-inflammatory, and antioxidant effects [7]. It has been reported that bergenin reduced morphine-induced physical dependence through the suppression of oxidative stress [8]. Moreover, TNF-α-induced inflammation in human bronchial epithelial cells was inhibited by bergenin [9]. However, the effect of bergenin on psoriasis has not been reported yet. The effects of bergenin on oxidative stress and inflammation of TNF-α-induced keratinocytes were then investigated. The related mechanism might provide potential strategy for the prevention of psoriasis.

EXPERIMENTAL

Cell culture and treatment

Human immortalized keratinocytes (HaCaT) were acquired from Biovector (Beijing, China), and cultured in RPMI-1640 (Life Technologies, Auckland, New Zealand) with 10 % fetal bovine serum (Life Technologies) in a 37 °C incubator. HaCaT was treated with bergenin (Sigma-Aldrich, Milwaukee, WI, USA) at doses of 12.5, 25, 50, 100 or 200 μM for 24 h. HaCaT was also treated with 10 ng/mL TNF-α (Sigma-Aldrich) for 48 h, followed by treatment with 50, 100 or 200 μM bergenin for another 24 h.

Cell viability and ELISA assays

HaCaT cells were seeded into 96-well plates. Cells were treated with bergenin for 24 h, and then incubated with 10 μL CCK8 solution (Beyotime, Beijing, China) for 2 h. Absorbance at 450 nm was measured using microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). For ELISA assay, TNF-α-treated HaCaT with or without bergenin treatment was lysed in RIPA buffer (Beyotime), and the supernatant was then harvested using centrifugation at 12000 g for 1 h. Levels of IL-6, IL-8, MDA, SOD and iNOS were determined with commercial ELISA kits (Thermo Fisher Scientific).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Cells were HaCaT was lysed in TRIzol kit (Invitrogen, Carlsbad, CA, USA), and the isolated RNAs were then reverse-transcribed into cDNAs. The cDNAs were performed with PreTaq II kit (Takara, Dalian, Liaoning, China) for the qRT-PCR analysis of IL-6 and IL-8. Thermocycling conditions: 95 ˚C for 2 min, 40 cycles at 95 ˚C for 30 s, 60 ˚C for 1 min and 68 ˚C for 25 s, were used, as shown in Table 1. The expression of IL-6 and IL-8 were normalized to GAPDH using $2^{-\Delta\Delta Cq}$ method.

Western blot assay

Cells were lysed in radioimmunoprecipitation assay buffer (RIPA buffer) (Beyotime, Beijing, China), and then centrifuged at 12000 g for 1 hour to collect the protein samples. Samples were separated using 10 % SDS-PAGE, and then transferred onto nitrocellulose membranes. The membranes were blocked in 5 % bovine serum albumin, and probed with specific antibodies: anti-fibronectin and anti-iNOS and anti-GAPDH (1: 2500), anti-p-p65 and anti-p65 (1: 2500), anti-p-IκBα and anti-IκBα (1: 3500), anti-Nrf2, anti-HO-1 and anti-Keap-1 (1: 4500). Followed by washing through phosphate-buffered saline-Tween-20, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1: 5000, Abcam), and the immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich). All the antibodies were purchased from Abcam (Cambridge, MA, USA).

Table 1: Primers used for qRT-PCR

Gene	Forward	Reverse
IL-6	5'-AACGCCTGGAAGAAGATGCC-3'	5'-CTCAGGCTGAACTGCAGGAA-3'
IL-8	5'-TTTCTGCAGCTCTCTGTGAGG-3'	5'-CTGCTGTTGTTGTTGCTTCTC-3'
GAPDH	5'-TCAACGACCACTTTGTCAAGCAGAGT-3'	5'-GCTGGTGGTCCAGGGGTCTTACT-3'

Statistical analysis

All the data are expressed as mean ± SEM (n = 3), and analyzed using Student's t test or one-way analysis of variance (ANOVA) using SPSS 19.0 software. A *p* value of < 0.05 was considered as statistically significant.

RESULTS

Effect of bergenin on cell viability of HaCaT

Treatment with bergenin did not reduce the viability of HaCaT cells (Figure 1 B), suggesting that bergenin showed no cytotoxicity on HaCaT.

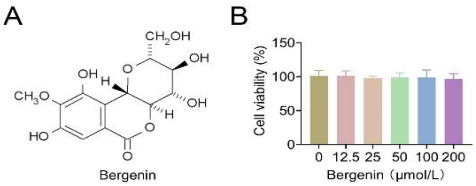

Figure 1: Effect of bergenin on cell viability of HaCaT. (A) Chemical structure of bergenin. (B) Treatment with bergenin did not reduce the viability of HaCaT cells

Effect of bergenin on inflammation of TNF-α-treated HaCaT

Treatment with TNF-α induced up-regulation of IL-6 and IL-8 in HaCaT (Figure 2 A and B). However, bergenin reduced the levels of IL-6 and IL-8 in TNF-α-treated HaCaT in a dosage dependent manner (Figure 2 A and B), demonstrating the anti-inflammatory effect against TNF-α-treated HaCaT.

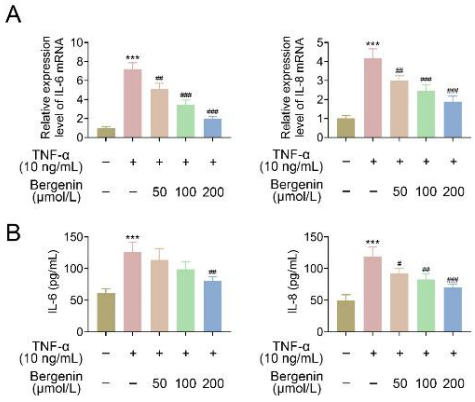

Figure 2: Effect of bergenin on inflammation of TNF-α-treated HaCaT. (A) Incubation with bergenin reduced mRNA expressions of IL-6 and IL-8 in TNF-α-treated HaCaT in a dose-dependent way. (B) Incubation with bergenin reduced protein expressions of IL-6 and IL-8 in TNF-α-treated HaCaT in a dose-dependent way. #*P* < 0.05, ##*p* < 0.01, ###, ***p* < 0.001

Effect of bergenin on oxidative stress of TNF-α-treated HaCaT

Treatment with TNF-α induced down-regulation of SOD, and up-regulation of MDA and iNOS in HaCaT cells (Figure 3 A). However, bergenin enhanced the level of SOD, and reduced the levels of MDA and iNOS in TNF-α-treated HaCaT cells in a dose dependent way (Figure 3 A). Moreover, bergenin attenuated TNF-α-induced increase in iNOS protein in HaCaT (Figure 3 B), demonstrating the antioxidant effect against TNF-α-treated HaCaT.

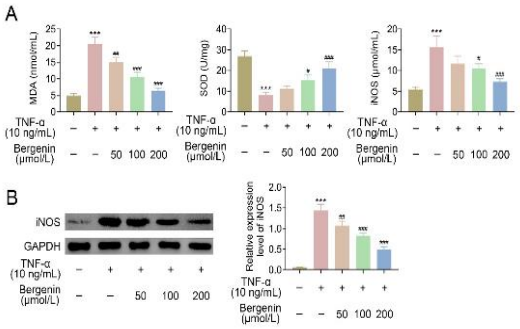

Figure 3: Effect of bergenin on oxidative stress of TNF-α-treated HaCaT. (A) Treatment with bergenin enhanced the level of SOD, and reduced the levels of MDA and iNOS in TNF-α-treated HaCaT in a dose-dependent manner. (B) Treatment with bergenin reduced protein expression of iNOS in TNF-α-treated HaCaT in a dose-dependent way. ***P* < 0.001 vs. control; #, ##, ###*p* < 0.05, *p* < 0.01, *p* < 0.001, respectively vs. TNF-α-treated HaCaT

Effect of bergenin on NF-κB and Nrf2 signalings in TNF-α-treated HaCaT

Protein expression of IκBα in HaCaT was decreased, while p-IκBα and p-p65 were increased by TNF-α (Figure 4). However, bergenin enhanced IκBα expression, while reducing the levels of p-IκBα and p-p65 in TNF-α-treated HaCaT (Figure 4). Moreover, bergenin attenuated TNF-α-induced increase in Keap-1, and the decreases in Nrf2 and HO-1 in HaCaT (Figure 4), indicating that bergenin promoted the inactivation of NF-κB and activation of Nrf2 signaling in TNF-α-treated HaCaT.

DISCUSSION

Traditional Chinese medicine has been clinically used in the treatment of psoriasis vulgaris, with long-term curative advantages [10]. This study found that bergenin, isolated from *Saxifraga stolonifera* Curt., also reduced inflammation and oxidative stress in psoriasis.

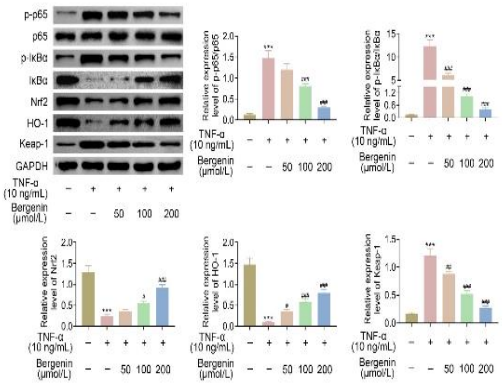

Figure 4: Effect of bergenin on NF-κB and Nrf2 signalings in TNF-α-treated HaCaT. Treatment with bergenin enhanced IκBα, Nrf2 and HO-1 expression, while reduced levels of p-IκBα, p-p65 and Keap-1 in TNF-α-treated HaCaT. #P < 0.05, ##p < 0.01, ###, ***p < 0.001

A previous study has shown that TNF-α induced increase in cell viability in HaCaT, and promoted the secretion of pro-inflammatory factors, including TNF-α, IFN-γ, IL-22, IL-1β, IL-4 and IL-6 [11]. Therefore, TNF-α-treated HaCaT is widely used as *in vitro* model of psoriasis [11]. TNF-α also induced inflammatory response in HaCaT through up-regulation of IL-6 and IL-8. Here, bergenin reduced the levels of IL-6 and IL-8 in TNF-α-treated HaCaT, thus suppressing TNF-α-induced inflammation in HaCaT. NF-κB was activated in TNF-α-treated HaCaT and important for immune and inflammation responses [12]. Eckol increased IκBα expression, while decreasd levels of p-IκBα and p-p65 in TNF-α-treated HaCaT to suppress the activation of NF-κB signaling [12]. Bergenin reduced inflammation of *Klebsiella pneumonia* infection through inactivation of NF-κB signaling [13]. This study also confirmed that bergenin attenuated TNF-α-induced decrease of IκBα, and increase of p-IκBα and p-p65 in HaCaT, thus promoting the inactivation of NF-κB signaling.

A previous study showed that total antioxidant capacity was down-regulated in psoriatic patients compared to the healthy subjects [14]. Moreover, accumulation of reactive oxygen species in psoriasis decreased the levels of antioxidants such as catalase, glutathione peroxidases and SOD, and increased the levels of hydrogen peroxide, hydroxyl radical and MDA, to promote activation of keratinocytes and immune cells through NF-κB signaling [5]. The activation of immune cells then promoted the secretion of pro-inflammatory factors and contributed to angiogenesis and keratinocyte over-proliferation [5]. Bergenin reduced the levels of MDA, iNOS, Gpx and nitrite in streptozotocin-induced mice, and exerted anti-oxidant effect against diabetic neuropathy [15]. This study showed that bergenin enhanced the level of SOD, and reduced the levels of MDA and iNOS in TNF-α-treated HaCaT, thereby contributing to the anti-oxidant effect against psoriasis.

Under oxidizing conditions, Nrf2 was activated and uncoupled from Keap-1, translocated into the nucleus and mediated the cytoprotective adaptive response by binding to the antioxidant response element of down-stream targets, such as glutamate-cysteine ligase catalytic subunit and HO-1 [16]. Nrf2 up-regulated HO-1 to reduce the inflammatory responses through inactivation of NF-κB signaling [16]. Curcumin promoted the activation of Keap1/Nrf2 signaling to ameliorate oxidative stress in TNF-α-treated HaCaT [17]. Bergenin up-regulated Nrf2 level and down-regulated NF-κB to prevent spatial memory deficit associated with neurodegeneration through antioxidant and anti-inflammatory effects [18]. This study also confirmed that bergenin attenuated TNF-α-induced increase of Keap-1, and decrease of Nrf2 and HO-1 in HaCaT, thus suppressing oxidative stress and inflammation in psoriasis.

CONCLUSION

Bergenin exerts antioxidant and anti-inflammatory effects against TNF-α-treated HaCaT through the activation of Nrf2 pathway and inactivation of NF-κB pathway. Therefore, bergenin is a potential agent for the treatment of psoriasis. However, the role of bergenin in an animal model of psoriasis should be investigated.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xin Ye and Haiming Ning designed the experiments, carried them out, analyzed and interpreted the data, Haiming Ning prepared the manuscript with contributions from all co-authors.

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REFERENCES

1. *Wang S, Lei J, Song X. Analysis of the effect of the Chinese herbal pair, Semen Persicae–Carthami Flos, on psoriasis using network pharmacology. Trop J Pharm Res 2021; 20(5): 973-979.*
2. *Kamiya K, Kishimoto M, Sugai J, Komine M, Ohtsuki M. Risk Factors for the Development of Psoriasis. Int J Mol Sci 2019; 20(18): 4347.*
3. *Benhadou F, Mintoff D, del Marmol V. Psoriasis: Keratinocytes or Immune Cells – Which Is the Trigger? Dermatology 2019; 235(2): 91-100.*
4. *Ni X, Lai Y. Keratinocyte: A trigger or an executor of psoriasis? J Leukocyte Biol 2020; 108(2): 485-491.*
5. *Xu F, Xu J, Xiong X, Deng Y. Salidroside inhibits MAPK, NF-κB, and STAT3 pathways in psoriasis-associated oxidative stress via SIRT1 activation. Redox Rep 2019; 24(1): 70-74.*
6. *Lin X, Meng X, Song Z. Homocysteine and psoriasis. Bioscience Rep 2019; 39(11): BSR20190867.*
7. *Ji Y, Wang D, Zhang B, Lu H. Bergenin Ameliorates MPTP-Induced Parkinson's Disease by Activating PI3K/Akt Signaling Pathway. J Alzheimers Dis 2019; 72: 823-833.*
8. *Yun J, Lee Y, Yun K, Oh S. Bergenin decreases the morphine-induced physical dependence via antioxidative activity in mice. Arch Pharm Res 2015; 38(6): 1248-1254.*
9. *Chen M, Chen C, Gao Y, Li D, Huang D, Chen Z, Zhao X, Huang Q, Wu D, Lai T, et al. Bergenin-activated SIRT1 inhibits TNF-α-induced proinflammatory response by blocking the NF-κB signaling pathway. Pulm Pharmacol Ther 2020; 62: 101921.*
10. *Guo X, Zhou D, Sun L, Wang P, Qu J, Zhang C, Wang Y, Chen Z, Li B, Hu J, et al. Traditional Chinese medicine for psoriasis vulgaris: A Protocol of a prospective, multicenter cohort study. Medicine 2020; 99(41): e21913-e21913.*
11. *Wu X, Deng X, Wang J, Li Q. Baicalin Inhibits Cell Proliferation and Inflammatory Cytokines Induced by Tumor Necrosis Factor α (TNF-α) in Human Immortalized Keratinocytes (HaCaT) Human Keratinocytes by Inhibiting the STAT3/Nuclear Factor kappa B (NF-κB) Signaling Pathway. Med Sci Monitor 2020; 26: e919392-e919392.*
12. *Cho S-H, Kim H-S, Lee W, Han EJ, Kim S-Y, Fernando IPS, Ahn G, Kim K-N. Eckol from Ecklonia cava ameliorates TNF-α/IFN-γ-induced inflammatory responses via regulating MAPKs and NF-κB signaling pathway in HaCaT cells. Int Immunopharmacol 2020; 82: 106146.*
13. *Tang Q, Wang Q, Sun Z, Kang S, Fan Y, Hao Z. Bergenin Monohydrate Attenuates Inflammatory Response via MAPK and NF-κB Pathways Against Klebsiella pneumonia Infection. Front Pharmacol 2021; 12: 651664-651664.*
14. *Barygina VV, Becatti M, Soldi G, Prignano F, Lotti T, Nassi P, Wright D, Taddei N, Fiorillo C. Altered redox status in the blood of psoriatic patients: involvement of NADPH oxidase and role of anti-TNF-α therapy. Redox Rep 2013; 18(3): 100-106.*
15. *Villarreal CF, Santos DS, Lauria PSS, Gama KB, Espírito-Santo RF, Juiz PJL, Alves CQ, David JM, Soares MBP. Bergenin Reduces Experimental Painful Diabetic Neuropathy by Restoring Redox and Immune Homeostasis in the Nervous System. Int J Mol Sci 2020; 21(14): 4850.*
16. *Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2-Keap1 signaling in oxidative and reductive stress. Biochim Biophys Acta Mol Cell Res 2018; 1865(5): 721-733.*
17. *Youn GS, Kwon D-J, Ju SM, Choi SY, Park J. Curcumin ameliorates TNF-α-induced ICAM-1 expression and subsequent THP-1 adhesiveness via the induction of heme oxygenase-1 in the HaCaT cells. BMB Rep2013; 46(8): 410-415.*
18. *Shal B, Khan A, Khan AU, Ullah R, Ali G, Islam SU, Haq IU, Ali H, Seo E-K, Khan S. Alleviation of Memory Deficit by Bergenin via the Regulation of Reelin and Nrf-2/NF-κB Pathway in Transgenic Mouse Model. Int J Mol Sci 2021; 22(12): 6603.*