Journal of Pharmacological Sciences 143 (2020) 209–218

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full Paper

Terrein suppressed lipopolysaccharide-induced neuroinflammation through inhibition of NF-κB pathway by activating Nrf2/HO-1 signaling in BV2 and primary microglial cells

Kwan-Woo Kim [a, 1], Hye Jin Kim [a, 1], Jae Hak Sohn [b], Joung Han Yim [c], Youn-Chul Kim [a], Hyuncheol Oh [a, *]

[a] Institute of Pharmaceutical Research and Development, College of Pharmacy, Wonkwang University, Iksan, 54538, Republic of Korea
[b] College of Medical and Life Sciences, Silla University, Busan, 46958, Republic of Korea
[c] Korea Polar Research Institute, KORDI, Yeonsu-gu, Incheon, 21990, Republic of Korea

ARTICLE INFO

Article history:
Received 2 July 2019
Received in revised form
6 December 2019
Accepted 7 January 2020
Available online 27 February 2020

Keywords:
Terrein
Anti-neuroinflammatory effects
NF-κB
HO-1
Nrf2

ABSTRACT

In the course of our continuous investigation on the bioactive marine-derived fungal metabolites, terrein was isolated from marine-derived fungal strain *Penicillium* sp. SF-7181. Terrein inhibited the over-production of pro-inflammatory mediators, such as nitric oxide (NO) and prostaglandin E2 (PGE_2), as well as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in lipopolysaccharide (LPS)-stimulated BV2 and primary microglial cells. This compound also repressed the LPS-induced production of pro-inflammatory cytokines, interleukin (IL)-1β and IL-6. These inhibitory effects of terrein were associated with the inactivation of the nuclear factor kappa B (NF-κB) pathway through suppression of the translocation of p65/p50 heterodimer into the nucleus, the phosphorylation and degradation of inhibitor kappa B (IκB)-α and the DNA binding activity of the p65 subunit. In addition, terrein induced the protein expression of heme oxygenase (HO)-1 through the activation of nuclear transcription factor erythroid-2 related factor 2 (Nrf2) in BV2 and primary microglial cells. The anti-inflammatory effect of terrein was blocked by pre-treatment with a selective HO-1 inhibitor, suggesting that its anti-neuroinflammatory effect is mediated by HO-1 induction.

1. Introduction

Glial cells and neuronal cells are components of the central nervous system (CNS) and the peripheral nervous system (PNS). Glial cells include oligodendrocytes, astrocytes, ependymal cells, and microglia, and the ratio of microglia is approximately 15% among total glial cells.[1] Microglia are a type of mononuclear macrophage of the brain, and play important roles in immune defense and tissue repair responses in the CNS.[2] Under pathological conditions in the brain, microglia are activated and extensively release various pro-inflammatory mediators including nitric oxide (NO), prostaglandins, and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.[3,4] Neuroinflammation mediated by microglia activation has been implicated in the development of neurological disorders, including trauma, stroke, brain infections, ischemia, and neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS).[3] Therefore, the suppression of neuroinflammation may have therapeutic and preventive potential in the pathogenesis of neurodegenerative diseases.

In the course of searching for bioactive metabolites from marine-derived fungal strains, terrein was isolated from an extract of the cultured fungal strain *Penicillium* sp. SF-7181 (Fig. 1A). This study describes the anti-neuroinflammatory effects of terrein in lipopolysaccharide (LPS)-stimulated BV2 and rat-derived primary microglial cells. Terrein was discovered as a secondary fungal metabolite from *Aspergillus terreus* by Raistrick and Smith in 1935,[5] and has been reported to occur in the genera *Aspergillus* (*fischeri*,[6] *stellatus*,[7] *pulvinus*[8]), *Penicillium*,[9] *Phoma*,[10] and *Pestalotiopsis*.[11]

* Corresponding author. Fax: +82 63 852 8837.
E-mail address: hoh@wku.ac.kr (H. Oh).
Peer review under responsibility of Japanese Pharmacological Society.
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jphs.2020.01.011

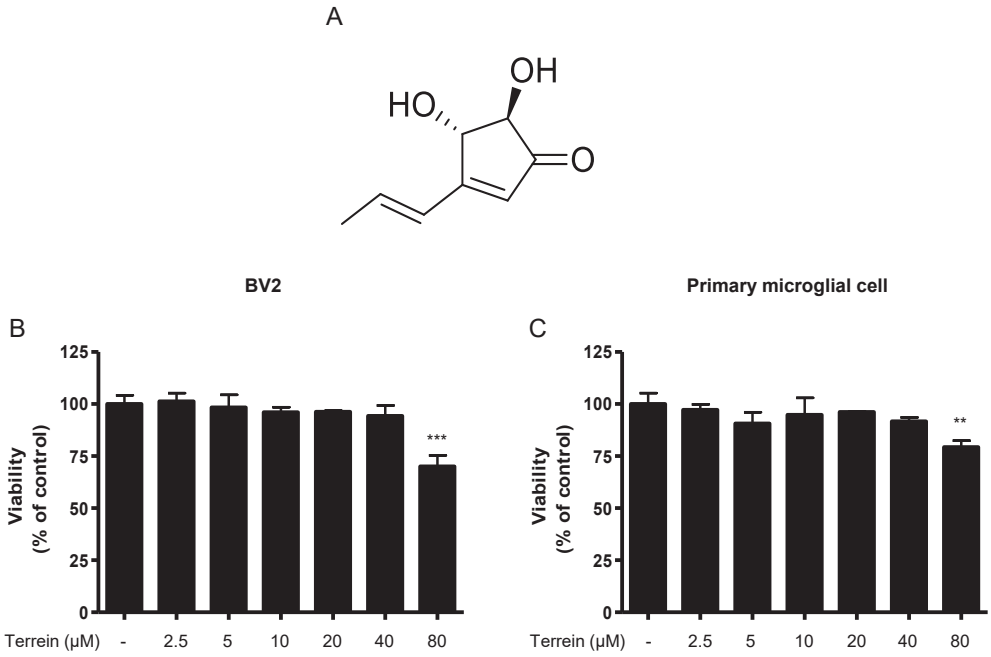

Fig. 1. (A) The chemical structure of terrein. (B, C) The effect of terrein on BV2 and primary microglial cell viability. Cells were treated with terrein at the indicated concentrations for 24 h, and cell viability was determined by MTT assay. $^{**}p < 0.01$, and $^{**}p < 0.001$ in comparison with the control group.

Terrein has two stereogenic centers (C2 and C3), and the absolute stereochemistry of (+)-terrein as 2R, 3S was determined by Barton & Miller.[12] The structure of (+)-terrein encountered in this study was elucidated based on analysis of the mass spectrometry (MS), specific rotation, and nuclear magnetic resonance (NMR) data, along with a comparison of these data with those in the literature (Fig. 1A).[13]

Secondary metabolites from marine-derived fungi have contributed to the development of novel classes of drug candidates.[14] Although terrein was discovered many years ago, few studies had been conducted with respect to its pharmacological activities. Terrein has been reported to suppress inflammation in human dental pulp cells through nuclear factor kappa B (NF-κB) inactivation by blocking the activation of protein kinase B (Akt).[15] Another study demonstrated that terrein inhibits age-related inflammation in oxidative stress-induced aged human diploid fibroblast (HDF) cells by increasing cell viability against hydrogen peroxide (H_2O_2) stress, and decreasing inflammatory molecules such as intracellular adhesion molecule (ICAM)-1, cyclooxygenase (COX)-2, IL-1β, and TNF-α.[16] Synthetic (+)-terrein represses IL-6-induced vascular endothelial growth factor (VEFG) secretion, and its inflammatory disease progression in human gingival fibroblasts.[17] However, the anti-neuroinflammatory effects of terrein have not yet been investigated, and to the best of our knowledge, this study would be the first report on the anti-neuroinflammatory effects of terrein.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were recorded using a Jasco P-2000 digital polarimeter. 1D and 2D NMR spectra were recorded using a JEOL JNM ECP-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). HRESIMS data were obtained using an electrospray ionization quadrupole-time of flight tandem mass spectrometry system (AB SCRIEX Triple).

2.2. Fungal materials, fermentation, extraction, and isolation of fungal metabolites

Penicillium sp. SF-7181 was isolated from sediment collected from the Ross Sea, Antarctica in January 2015. One gram of the sample was ground with a mortar and pestle, and mixed with sterile seawater (10 mL). A portion (0.1 mL) of the sample was processed utilizing the spread plate method in potato dextrose agar (PDA) medium containing seawater and incubated at 25 °C for 14 days. After sub-culturing the isolates several times, the final pure cultures were selected and stored at −75 °C. The fungal strain SF-7181 was identified based on the analysis of the ITS gene sequence. A GenBank search with the ITS gene of SF-7181 (GenBank accession number MK300684) indicated *Penicillium cairnsense* (JN617669), *Penicillium quebecense* (JN617661), *Penicillium neomiczynskii* (JX230996), and *Penicillium cosmopolitanum* (MH864384) as the closest matches showing sequence identities of 100%, 99.83%, 99.83%, and 99.5%, respectively. Therefore, the marine-derived fungal strain SF-7181 was characterized as *P. cairnsense*. The fungal strain SF-7181 was cultured in a Fernbach-style flask containing 150 mL of PDA with 3% NaCl. The flask was incubated at 25 °C for 14 days. The culture flask was extracted with 1 L of ethyl acetate (EtOAc), and the resulting organic phase was then evaporated *in vacuo* to yield the EtOAc extract of SF-7181 (121.6 mg). Then, 20 mg of EtOAc extract was separated by semi-preparative HPLC and eluted with a gradient of 20–100% MeOH in H_2O over 30 min to give SF-7181-2 (6.8 mg, $t_R = 14$ min).

2.2.1. (+)-Terrein

Colorless solid; $[\alpha]_D^{22}$ +103.8 (c 0.14, H_2O); ^{1}H NMR (CD_3OD, 400 MHz) δ 6.83 (1H, m, H-7), 6.43 (1H, d, J = 15.8 Hz, H-6), 6.00 (1H, s, H-5), 4.66 (1H, d, J = 2.7 Hz, H-3), 4.06 (1H, d, J = 2.7 Hz, H-2), 1.94 (3H, dd, J = 1.5, 6.8 Hz, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ 205.6 (C-1), 170.9 (C-4), 141.8 (C-7), 126.4 (C-6), 125.9 (C-5), 82.4 (C-2), 78.1 (C-3), 19.4 (C-8); HRESIMS m/z 155.0709 $[M + H]^+$ (calculated for $C_8H_{11}O_3$, 155.0708).

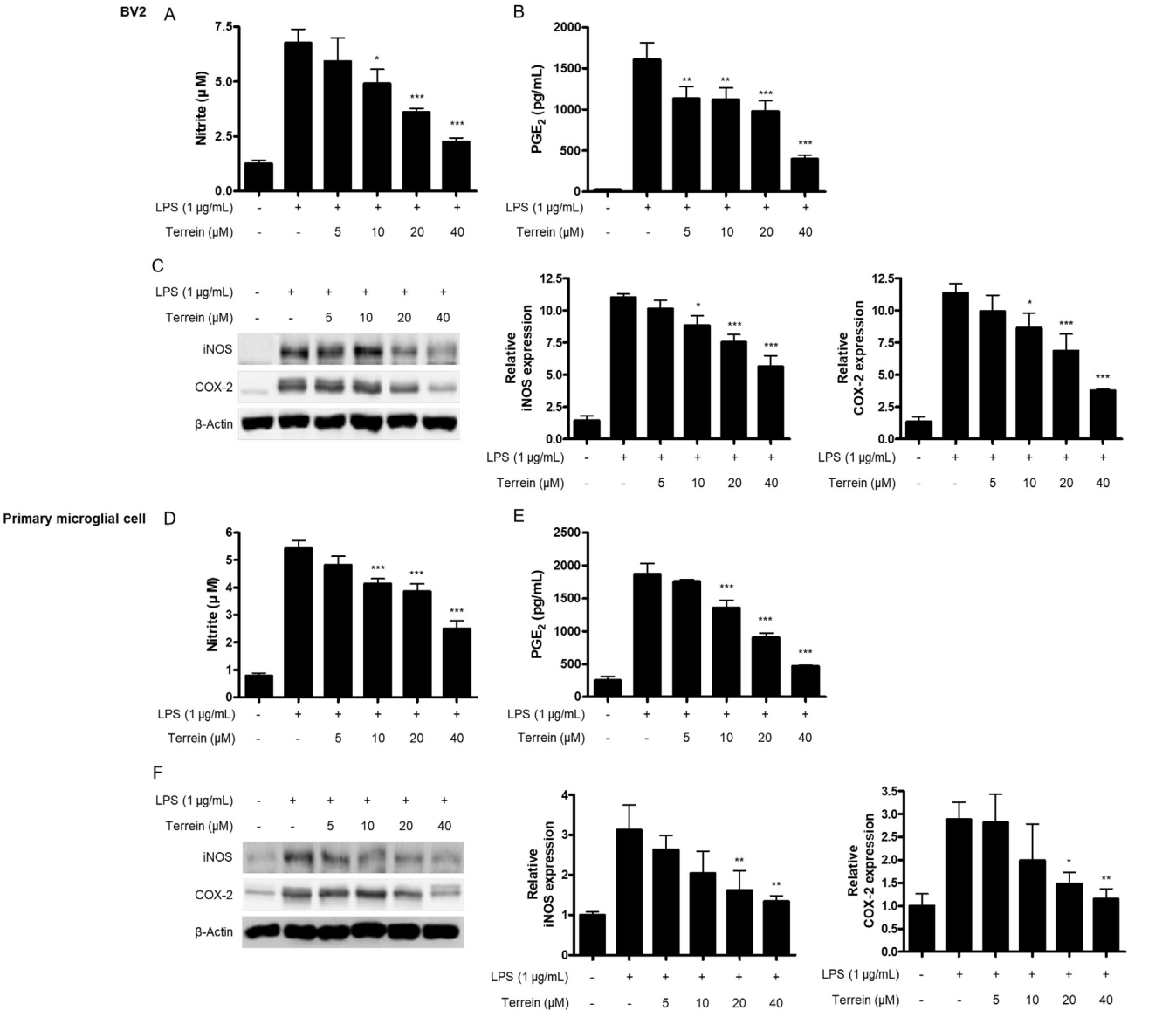

Fig. 2. (A, B) Effects of terrein on the LPS-induced production of nitrite and PGE_2. Cells were pretreated with or without the indicated concentration of terrein for 3 h and then stimulated with LPS (1 μg/mL) for 24 h. Nitrite levels were determined using the Griess reaction and PGE_2 was quantified by ELISA. Values shown are means ± SD of three independent experiments. $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ in comparison with the LPS group. (C) Effects of terrein on the LPS-induced expression of iNOS and COX-2. Lysates were prepared from cells pre-treated with or without the indicated concentrations of terrein for 3 h and then with LPS (1 μg/mL) for 24 h. The level of iNOS and COX-2 protein expression was determined by Western blot analysis. Representative blots from three independent experiments are shown. $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ in comparison with the LPS group.

2.3. Chemicals and reagents

Details of chemicals and reagents used in this investigation were described in our previous report.[18] LPS (from *Escherichia coli*, serotype 055:B5) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.4. BV2 cell culture and isolation of primary microglial cells

BV2 microglial cells were cultured and rat primary microglial cells were isolated based on our previous reported protocols.[19]

2.5. MTT assay for cell viability

BV2 and primary microglial cells were seeded at 1×10^5 cells/mL and treated with terrein for 24 h. MTT assays were conducted to determine BV2 and primary microglial cell viability based on the previously reported protocol.[20]

2.6. Determination of nitrite

The nitrite concentration in the culture medium, an indicator of NO production, was measured using the Griess reaction. The detailed procedures for this assay are described in our previous report.[20]

2.7. PGE_2, IL-1β, IL-6, and TNF-α assay

The culture media were collected to determine the levels of PGE_2, IL-1β, IL-6, and TNF-α present in each sample using the appropriate ELISA kit from R&D Systems Inc. (Minneapolis, MN, USA). Three independent assays were performed according to the manufacturer's instructions.

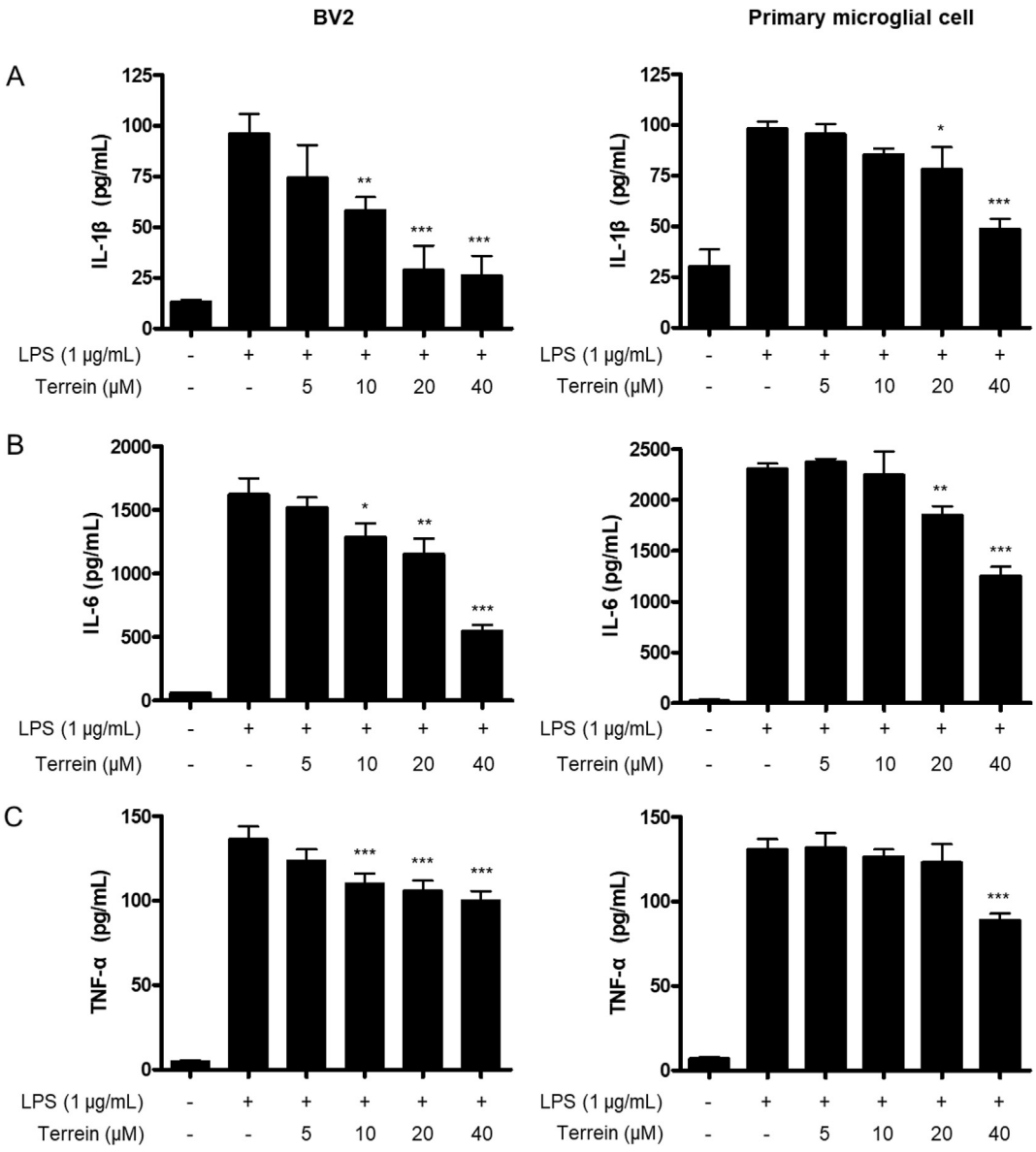

Fig. 3. Effects of terrein on the production of LPS-induced pro-inflammatory cytokines in BV2 and primary microglial cells. Cells were pretreated with or without the indicated concentration of terrein for 3 h and then stimulated with LPS (1 μg/mL) for 24 h. The concentration of cytokines was measured by ELISA. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ in comparison with the LPS-treated group.

2.8. Western blot analysis

Western blot analysis was performed as described previously.[19]

2.9. Preparation of cytosolic and nuclear fractions

The cytosolic and nuclear fractions were extracted using the Cayman Nuclear extraction kit from Cayman (Ann Arbor, MI, USA), and each fraction was lysed according to the manufacturer's instructions.

2.10. DNA binding activity

The DNA binding activity of NF-κB and Nrf2 in nuclear extracts was measured using NF-κB transcription factor assay kits and Nrf2 transcription factor assay kits from Cayman (Ann Arbor, MI, USA) respectively, according to the manufacturers' instructions. Three independent replicates were performed.

2.11. Nrf2 siRNA transfection

Nrf2-targeted siRNA was used to knockout Nrf2. The detailed procedure was described in our previous report.[18]

2.12. Statistical analysis

The data are expressed as the mean ± standard deviation (SD) of at least three independent experiments. To compare three or more groups, a one-way analysis of variance (ANOVA) was used, followed by Tukey's multiple comparison tests. Statistical analysis was performed using GraphPad Prism software, version 3.03 (GraphPad Software Inc., San Diego, CA, USA).

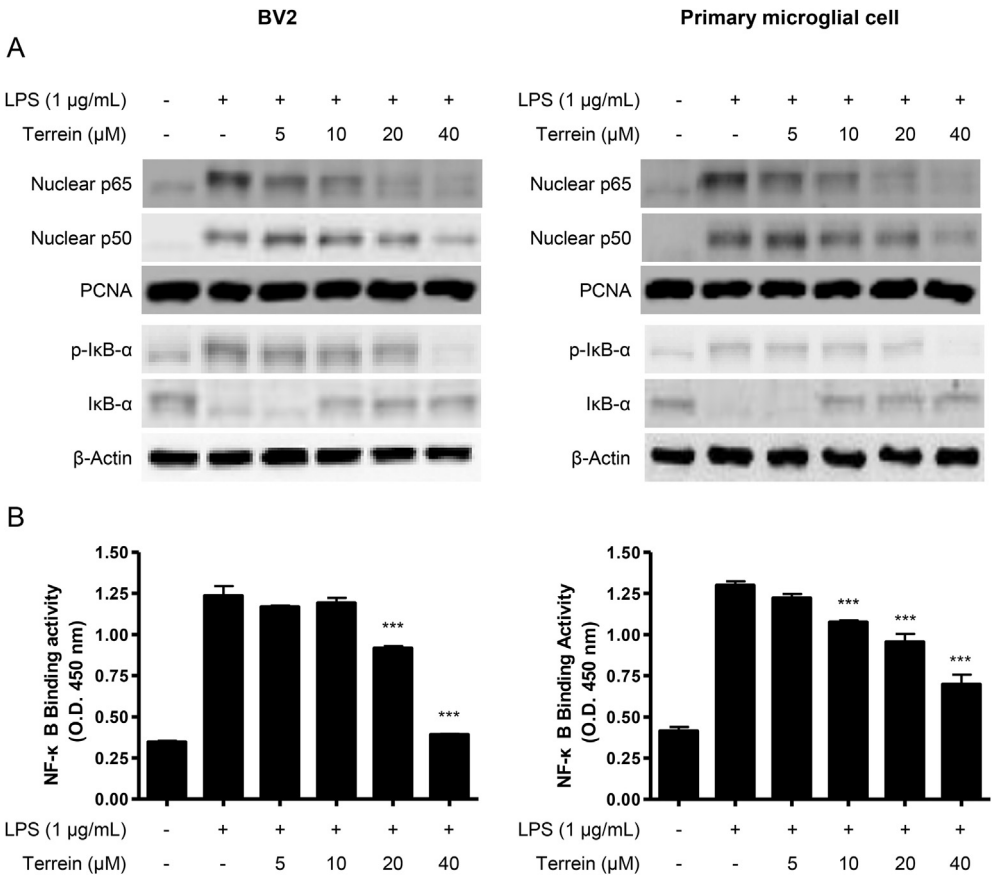

Fig. 4. Effects of terrein on the LPS-induced activation of NF-κB in BV2 and primary microglial cells. Cells were pre-treated with the indicated concentrations of terrein for 12 h and then stimulated with LPS (1 μg/mL) for 1 h. (A) Nuclear and cytosolic extracts were isolated and the levels of p65 and p50 in the nuclear fraction and p-IκB-α and IκB-α in the cytosolic fraction were determined by Western blot analysis. The experiment was repeated three times, and similar results were obtained. PCNA and β-actin were used as internal controls. (B) NF-κB ELISA kit was used to determine the degree of NF−κB binding in nuclear extracts. Values are means ± SD of three independent experiments. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ in comparison with the LPS group.

3. Results

3.1. Effect of terrein on the viability of BV2 and primary microglial cells

To evaluate the cytotoxicity and to determine the appropriate dose range of terrein for the study, we performed an MTT assay to evaluate the viability of BV2 and primary microglial cells treated with terrein. As shown in Fig. 1B and C, the non-cytotoxic concentration range was determined to be 0.0−40 μM of terrein.

3.2. Effect of terrein on the LPS-induced upregulation of inflammatory mediators in BV2 and primary microglial cells

To investigate the inflammation regulatory effect of terrein, the levels of various inflammatory mediators (NO, PGE_2, iNOS, COX-2, IL-1β, IL-6, and TNF-α) in LPS-treated cells were analyzed by Griess reaction, ELISA, and Western blotting. Pre-treatment with terrein (5−40 μM) decreased LPS-induced NO and PGE_2 production as well as iNOS and COX-2 protein expression in BV2 and primary microglial cells (Fig. 2). As shown in Fig. 3, pre-treatment with terrein attenuated the production of IL-1β, IL-6, and TNF-α in both microglial cells.

3.3. Effect of terrein on the LPS-induced activation of NF-κB in BV2 and primary microglial cells

Subsequently, the effect of terrein on the LPS-induced activation of the NF-κB pathway was examined. Stimulation with LPS increased the nuclear accumulation of NF-κB subunits (p65 and p50) in a nuclear faction, and the phosphorylation and degradation of IκB-α in cytosolic fraction in BV2 and primary microglial cells. However, pre-treatment with terrein attenuated all these responses (Fig. 4A). In addition, the DNA binding activity of NF-κB protein p65 increased in LPS-stimulated cells, but this activity was attenuated upon pre-treatment with terrein in BV2 and primary microglial cells (Fig. 4B).

3.4. Effect of terrein on the activation of HO-1/Nrf2

We further investigated whether the anti-neuroinflammatory effect of terrein is involved in the activation of the HO-1/Nrf2 signaling pathway. Treatment with terrein for 12 h induced the expression of HO-1 protein in both BV2 and primary microglial cells in a dose-dependent pattern (Fig. 5A). In addition, the levels of Nrf2 decreased in cytosol, and increased in nuclear following treatment with terrrein for 4 h, indicating that terrein activates the Nrf2 pathway (Fig. 5B). Furthermore, terrein increased the DNA binding

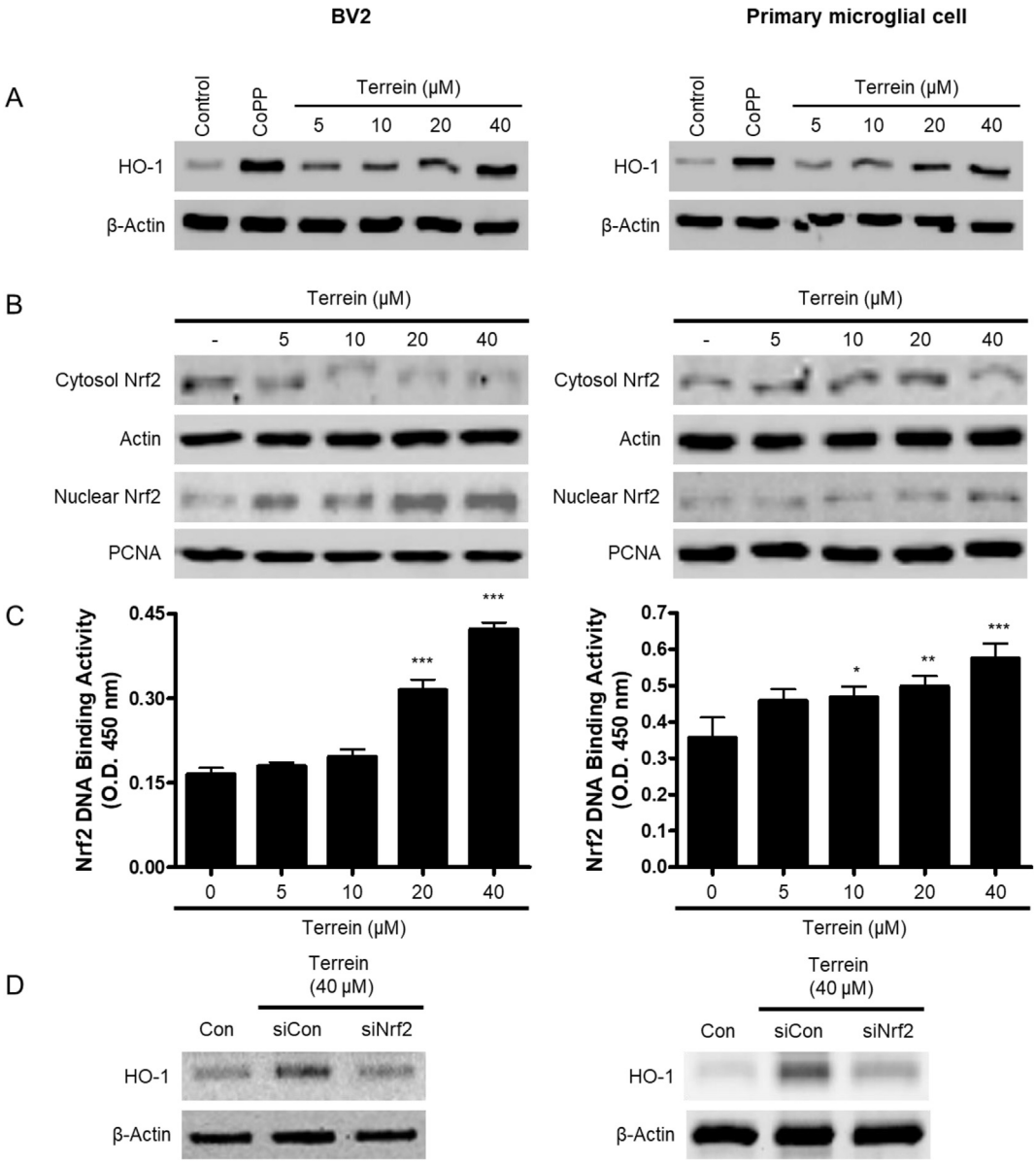

Fig. 5. Effects of terrein on the activation of HO-1/Nrf2 pathway in BV2 and primary microglial cells. (A) Cells were treated with terrein for 12 h at various concentrations and Western blot analysis was performed for HO-1 expression. The HO-1 inducer, CoPP, was used as a positive control. Representative blots of three independent experiments are shown. (B) Cells were treated with various concentrations of terrein for 4 h, and the levels of Nrf2 protein in nuclear and cytoplasm fractions were determined by Western blot analysis. The experiment was repeated three times and similar results were obtained. (C) Nrf2 transcription factor assay kit was used to determine the degree of Nrf2 DNA binding in nuclear extracts. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ in comparison with the control group. (D) Cells were transiently transfected with control siRNA and Nrf2 siRNA for 24 h, and then treated with or without 40 μM terrein. Levels of HO-1 protein in each lysate were determined using Western blot analysis. Experiments were repeated three times and similar results were obtained. PCNA and β-actin were used as internal controls.

activity of Nrf2 in a dose-dependent manner (Fig. 5C). When BV2 and primary microglial cells were transfected with siRNA against Nrf2, terrein-induced upregulation of HO-1 protein was blocked (Fig. 5D).

3.5. Correlation of HO-1 upregulation by terrein with its anti-neuroinflammatory effects

To determine the role of terrain-induced HO-1 in the anti-neuroinflammatory effects observed in the LPS-stimulated cells, we examined the effects of SnPP, which is an established HO activity inhibitor, on LPS-induced inflammatory responses. Treatment with terrein decreased LPS-induced NO/PGE_2 production, iNOS/COX-2 expression, nuclear translocation of NF-κB p65, and the DNA binding activity of NF-κB p65. However, co-treatment with SnPP partially blocked these effects in both BV2 and primary microglial cells (Fig. 6). In addition, SnPP reversed the inhibitory effect of terrein on the LPS-induced production of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in both cells (Fig. 7.).

4. Discussion

This study indicates that terrein has anti-neuroinflammatory effects in LPS-stimulated BV2 and rat-derived primary microglial cells. The anti-neuroinflammatory activity of terrein was

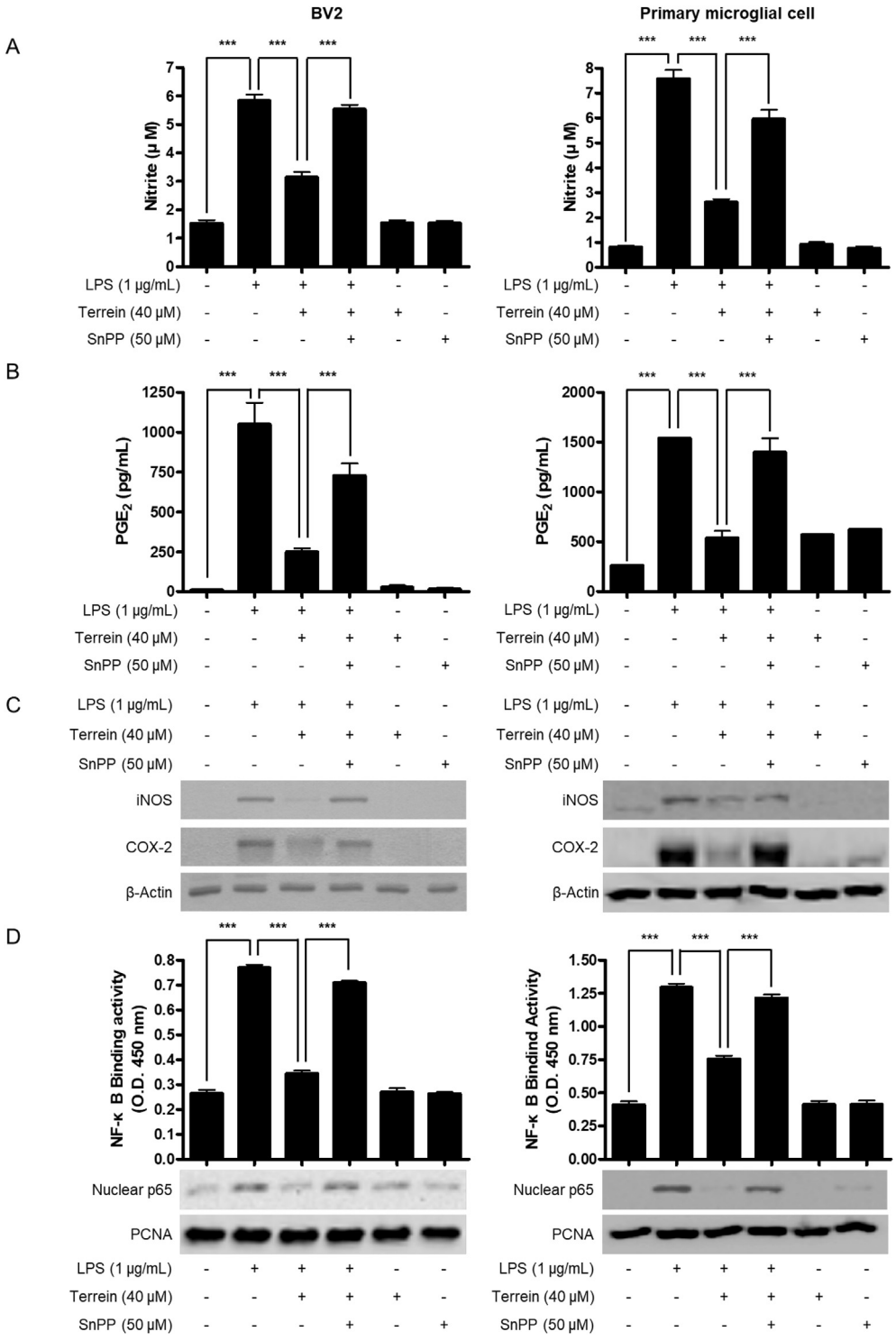

Fig. 6. Effects of SnPP on terrein-induced regulation of nitrite, PGE_2, iNOS, COX-2, and activation of NF-κB in BV2 and primary microglial cells. (A, B, C) Effects of SnPP on terrein-induced inhibitory effects of production of nitrite and PGE_2, and protein expression of iNOS and COX-2. Cells were pre-treated with 50 μM SnPP for 1 h, incubated with or without 40 μM of terrein for 3 h, and then stimulated with LPS (1 μg/mL) for 24 h. Nitrite levels were determined using Griess reaction, and PGE_2 was quantified using ELISA. The levels of iNOS and COX-2 were determined by Western blot analysis. Values are means ± SD of three independent experiments. β-Actin was used as an internal control. ***$p < 0.001$. (D) Effects of SnPP on terrein-induced inactivation of NF-κB. Cells were pre-treated with 50 μM SnPP for 1 h, incubated with or without 40 μM of terrein for 12 h, and then stimulated with LPS (1 μg/mL) for 1 h. The levels of nuclear p65 were determined using Western blot analysis. A NF-κB ELISA kit was used to determine the degree of NF−κB binding in nuclear extracts. PCNA was used as the internal control. All experiments were repeated three times and similar results were obtained.

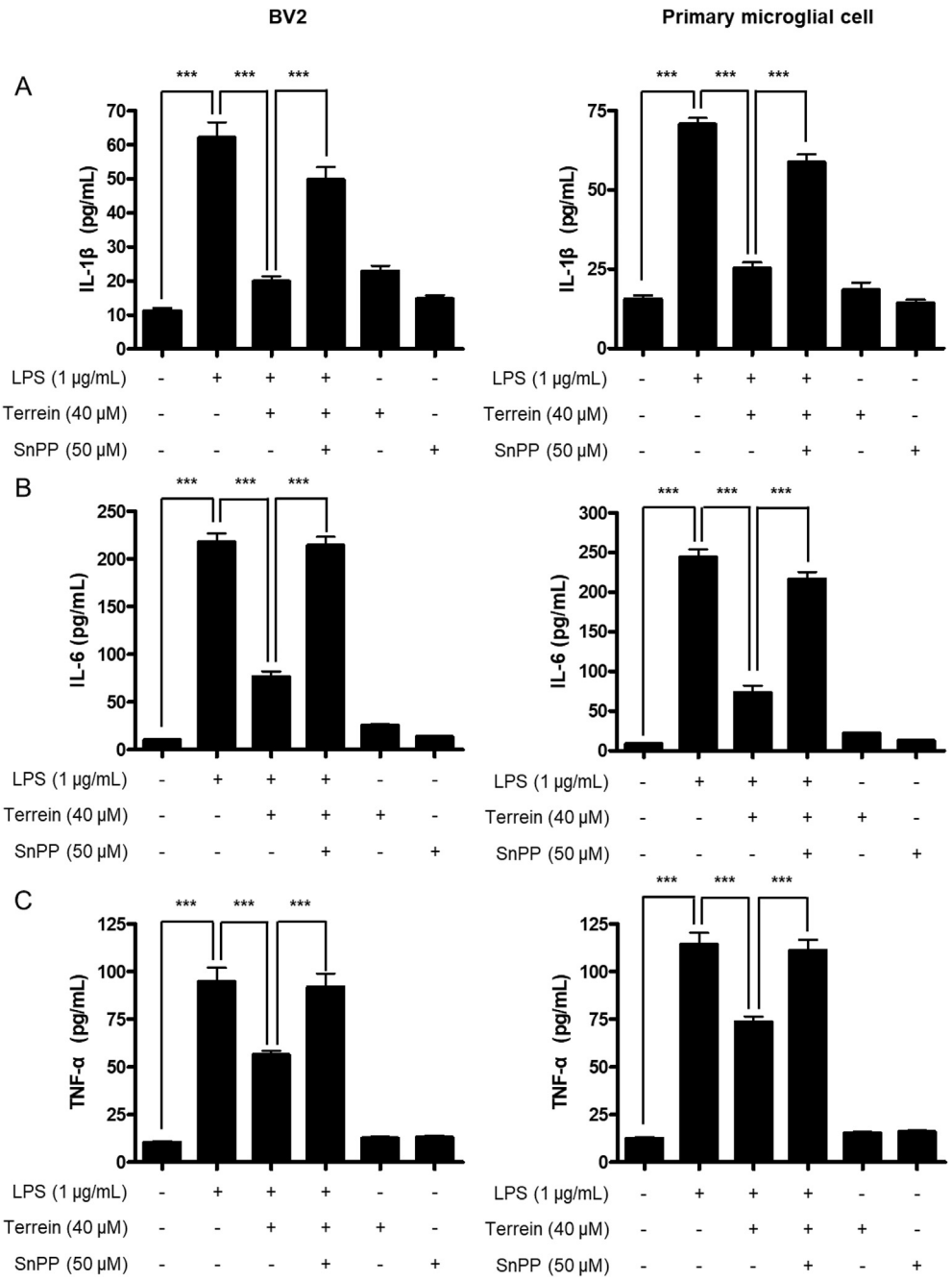

Fig. 7. Effects of SnPP on terrein-induced production of pro-inflammatory cytokines in BV2 and primary microglial cells. Cells were pre-treated with 50 μM SnPP for 1 h, incubated with or without 40 μM of terrein for 3 h, and then stimulated with LPS (1 μg/mL) for 24 h. The concentration of cytokines was measured by ELISA. ***$p < 0.001$.

correlated with the inhibition of NF-κB pathway. In addition, terrein was shown to activate the HO-1/Nrf2 signaling pathway, and this response could contribute to inactivation of NF-κB pathway.

In this study, BV2 cell line and rat-derived microglial cells were employed to evaluate the anti-neuroinflammatory effects of terrein. The BV2 cell line is derived from v-raf/v-myc-immortalized murine primary microglia, and primary microglial cells are isolated from the cortex of rats or mice. These cells can be activated by various stimulants including LPS, interferon, or β-amyloid, whereupon they release pro-inflammatory mediators.[21] Therefore, these cells have been mostly used as *in vitro* models for the investigation of cellular functions, pharmacologic and physiologic features in microglial cells.[21,22] Nevertheless, BV2 cells cannot mimic all characteristics of primary microglial cells. Primary microglial cells can react more sensitively to LPS and exhibit greater responses as compared to BV2 cells with regard to migration, expression of activation markers, and production and secretion of inflammatory mediators.[21,23,24] Moreover, cultured primary microglial cells retain the majority of *in vivo* physiological functions of activated microglia, thus they have been regarded as an *in vitro* model more appropriate than BV2 cells for investigating the pharmacological and neurological characteristics of microglial cells.[21,22] Therefore, the anti-neuroinflammatory effects of terrein were elucidated in not only BV2 immortalized cells but also in primary microglial cells.

Activated microglial cells can induce the secretion of pro-inflammatory mediators such as NO, PGE_2, iNOS, COX-2, and cytokines including IL-1β, IL-6, and TNF-α.[25,26] The expression of iNOS and COX-2 is responsible for the production of NO and PGE_2 respectively. These enzymes and byproducts have a basic role in immune response in various immune cells such as macrophages, microglia, and astrocytes.[27] In addition, excessive production of pro-inflammatory cytokines exacerbates inflammatory responses in the CNS, and contributes to the pathogenesis of neuroinflammation in neurodegenerative diseases.[28] Previous studies have shown the anti-neuroinflammatory effects of some natural products through the inhibition of pro-inflammatory cytokines.[28,29] Therefore, the inactivation of these pro-inflammatory mediators could be regarded as one of the targets for treatment of neuroinflammatory diseases. The current study sought to elucidate the anti-neuroinflammatory effects of terrein in LPS-induced BV2 and primary microglial cells. The overproduction of NO/PGE_2 and the protein expression of iNOS/COX-2 were repressed by pretreatment with terrein (Fig. 2). In addition, terrein decreased the overproduction of IL-1β, IL-6, and TNF-α cytokines in both LPS-stimulated BV2 and primary microglial cells (Fig. 3).

NF-κB is one of the most important transcription factors associated with immune responses, and plays a significant role in the induction of pro-inflammatory cytokine gene expression.[30] The NF-κB family consists of five proteins, p65, Rel B, c-Rel, p105/p50, and p100/p52. These subunits can form 15 different homodimeric and heterodimeric complexes, and the p65/p50 heterodimer is the most abundant dimer found in almost cell types.[31] Under normal conditions, these complexes are present in the cytoplasm of cells through binding to the IκB protein and remain inactive. Under condition of inflammatory response induced by LPS, IκB can be phosphorylated and degraded, and NF-κB dimers are then dissociated from IκB. The released NF-κB dimers translocate into the nucleus, bind to DNA at the κB site, and induce the expression of inflammation-related genes.[28] The abnormal regulation of the NF-κB pathway in microglia is involved in the development of pathological conditions such as autoimmune encephalomyelitis, ischemia, and Alzheimer's disease.[32] In the current study, pretreatment with terrein downregulated the LPS-induced translocation of p65/p50 subunits into the nucleus, phosphorylation and degradation of IκB, and DNA binding activity of the p65 subunit (Fig. 4). Thus, our data showed that the inhibitory effect of terrein on neuroinflammatory responses was mediated by inactivating the NF-κB pathway. Several agents that can block NF-κB activation have potential immunosuppressive effects. Dexamethasone and leflunomide metabolite were reported as a novel immunosuppressive and anti-inflammatory agent, and these compounds inhibited NF-κB activation in a human T cell line.[33,34] Terrein showed complete inhibition of NF-κB signaling in this study, therefore this compound could also have the possible effects of the immunosuppression.

Microglia can express various detoxifying or antioxidant enzymes including HO-1, NAD(P)H:quinone oxidoreductase 1 (NQO1), peroxiredoxin (PRX), and thioredoxin (Trx), and can prevent damages to neighboring neurons by regulating Nrf2, a member of the NF-E2 family of the basic leucin zipper transcription factors.[4] Under normal conditions, the activity of Nrf2 is repressed in the cytoplasm by forming a complex with Kelch-like ECH-associated protein 1 (Keap1). Under cellular stress or inflammatory conditions, Nrf2 dissociates from Keap1, and translocates into the nucleus. In the nucleus, Nrf2 binds to an antioxidant response elements (ARE) site on the DNA, and accelerates the expression of various cytoprotective genes.[4] Especially, HO-1 is one of the inducible enzymes mediated by upregulation of the Nrf2/ARE pathway, and is involved in a variety of regulatory and protective cellular mechanisms.[35] HO-1 can catalyze the conversion of heme to biliverdin, ferrous ion (Fe^{2+}), and carbon monoxide, which have been shown to have antioxidant and anti-inflammatory activities.[4] Various reports have demonstrated that HO-1 expression and its related signaling pathways induce anti-inflammatory effects.[1,4] Our recent studies also showed that HO-1 induction inhibits the neuroinflammatory responses in microglial cells.[18,19] In the present study, we explored the effects of terrein on the activation of the Nrf2/HO-1 pathway. Our results showed that terrein induced HO-1 protein, and this response was associated with the nuclear translocation of Nrf2 (Fig. 5). A selective inhibitor of HO activity (SnPP) reversed the anti-neuroinflammatory effects of terrein, which are inhibition of NO/PGE_2 production, iNOS/COX-2 expression, nuclear translocation of p65, and DNA binding activity of p65 (Fig. 6). Taken together, our data demonstrate that terrein upregulated Nrf2 signaling and subsequently induced HO-1 protein, which were correlated to its anti-neuroinflammatory effects.

Conclusion

In this study, our chemical investigation of the marine-derived fungal isolate *Penicillium* sp. SF-7181 resulted in the isolation and identification of secondary metabolite, terrein. Terrein showed anti-neuroinflammatory effects by inhibiting the production of NO, PGE_2, and pro-inflammatory cytokines, and the protein expression of iNOS and COX-2 through inactivation of the NF-κB pathway. In addition, terrein-induced activation of Nrf2/HO-1 pathway was related to the anti-neuroinflammatory effects of terrein. Thus, terrein may serve as a potential candidate for the development of preventive agents to treat neurodegenerative diseases.

Declaration of Competing Interest

The authors indicated no potential conflicts of interest.

Acknowledgement

This work was supported by a grant from the Korea Polar Research Institute (PE20010).

References

1. Lin HY, Huang BR, Yeh WL, et al. Antineuroinflammatory effects of lycopene via activation of adenosine monophosphate-activated protein kinase-α1/heme oxygenase-1 pathways. *Neurobiol Aging*. 2014;35(1):191–202.
2. Prinz M, Priller J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat Rev Neurosci*. 2014;15(5):300–312.
3. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*. 2007;8:57–69.
4. Kim J, Lim J, Kang BY, Jung K, Choi HJ. Capillarisin augments anti-oxidative and anti-inflammatory responses by activating Nrf2/HO-1 signaling. *Neurochem Int*. 2017;105:11–20.
5. Raistrick H, Smith G. Studies in the biochemistry of micro-organisms: the metabolic products of *Aspergillus terreus* Thom. A new mould metabolic product-terrein. *Biochem J*. 1935;29(3):606–611.
6. Misawa M, Nara T, Nakayama K, Kinoshita S. Formation of terrein by *Aspergillus fischeri* Wehmer. *Nippon Nogeikagaku Kaishi*. 1962;36(8):699–703.
7. Qureshi IH, Kamal A, Noorani R, Aziz S, Husain SA. Studies in the biochemistry of microorganisms. Part VII. Terrein and kojic acid; metabolic products of *Aspergillus stellatus* Curzi. *Pakistan J Sci Ind Res*. 1968;11(4):367–369.
8. Qureshi IH, Begum T, Noorani R. Isolation and identification of the metabolic products of *Aspergillus pulvinus* Kwon and Fennel. Comparative studies of production of terrein and ergosterol in different media. *Pakistan J Sci Ind Res*. 1976;19(3–4):120–122.
9. Kim WG, Ryoo IJ, Park SH, et al. Terrein, a melanin biosynthesis inhibitor, from *Penicillium* sp. 20135. *J Microbiol Biotechnol*. 2005;15(4):891–894.
10. Dunn AW, Entwistle ID, Johnstone RAW. Terrein and other metabolites of *Phoma* species. *Phytochemistry*. 1975;14(9):2081–2082.
11. Harper JK, Arif AM, Li JY, Strobel G, Grant DM. Terrein. *Acta Crystallogr*. 2000;C56(12):e570–e571.
12. Barton DHR, Miller E. The constitution and stereochemistry of terrein. *J Chem Soc*. 1955:1028–1029.

13. Al Trabolsy ZBK, Anouar EH, Zakaria NSS, et al. Antioxidant activity, NMR, X-ray, ECD and UV/vis spectra of (+)-terrein: experimental and theoretical approaches. *J Mol Struct*. 2014;1060:102–110.
14. Bugni TS, Ireland CM. Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Nat Prod Rep*. 2004;21(1):143–163.
15. Lee JC, Yu MK, Lee R, et al. Terrein reduces pulpal inflammation in human dental pulp cells. *J Endod*. 2008;34(4):433–437.
16. Lee YH, Lee SJ, Jung JE, Kim JS, Lee NH, Yi HK. Terrein reduces age-related inflammation induced by oxidative stress through Nrf2/ERK1/2/HO-1 signalling in aged HDF cells. *Cell Biochem Funct*. 2015;33(7):479–486.
17. Mandai H, Omori K, Yamamoto D, et al. Synthetic (+)-terrein suppresses interleukin-6/soluble interleukin-6 receptor induced-secretion of vascular endothelial growth factor in human gingival fibroblasts. *Bioorg Med Chem*. 2014;22(19):5338–5344.
18. Kim KW, Yoon CS, Kim YC, Oh H. Desoxo-narchinol A and Narchinol B isolated from *Nardostachys jatamansi* exert anti-neuroinflammatory effects by up-regulating of nuclear transcription factor erythroid-2-related factor 2/heme oxygenase-1 signaling. *Neurotox Res*. 2019;35(1):230–243.
19. Kim KW, Kim HJ, Sohn JH, Yim JH, Kim YC, Oh H. Anti-neuroinflammatory effect of 6,8,1'-tri-*O*-methylaverantin, a metabolite from a marine-derived fungal strain *Aspergillus* sp., via upregulation of heme oxygenase-1 in lipopolysaccharide-activated microglia. *Neurochem Int*. 2018;113:8–22.
20. Ko W, Sohn JH, Jang JH, et al. Inhibitory effects of alternaramide on inflammatory mediator expression through TLR4-MyD88-mediated inhibition of NF-κB and MAPK pathway signaling in lipopolysaccharide-stimulated RAW264.7 and BV2 cells. *Chem Biol Interact*. 2016;244:16–26.
21. Horvath RJ, Nutile-McMenemy N, Alkaitis MS, Deleo JA. Differential migration, LPS-induced cytokine, chemokine, and NO expression in immortalized BV-2 and HAPI cell lines and primary microglial cultures. *J Neurochem*. 2008;107(2):557–569.
22. Giulian D, Baker TJ. Characterization of ameboid microglia isolated from developing mammalian brain. *J Neurosci*. 1986;6(8):2163–2178.
23. Henn A, Lund S, Hedtjärn M, Schrattenholz A, Pörzgen P, Leist M. The suitability of BV2 cells as alternative model system for primary microglia cultures or for animal experiments examining brain inflammation. *ALTEX*. 2009;26(2):83–94.
24. Das A, Kim SH, Arifuzzaman S, et al. Transcriptome sequencing reveals that LPS-triggered transcriptional responses in established microglia BV2 cell lines are poorly representative of primary microglia. *J Neuroinflammation*. 2016;13(1):182.
25. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*. 2007;15(6):252–259.
26. Kim ME, Na JY, Park YD, Lee JS. Anti-neuroinflammatory effects of vanillin through the regulation of inflammatory factors and NF-κB signaling in LPS-stimulated microglia. *Appl Biochem Biotechnol*. 2018;187(3):884–893.
27. Koppal T, Petrova TV, Van Eldik LJ. Cyclopentenone prostaglandin 15-deoxy-Delta(12,14)-prostaglandin J(2) acts as a general inhibitor of inflammatory responses in activated BV-2 microglial cells. *Brain Res*. 2000;867(1–2):115–121.
28. Yu HY, Kim KS, Lee YC, Moon HI, Lee JH. Oleifolioside A, a New active compound, attenuates LPS-stimulated iNOS and COX-2 expression through the downregulation of NF-κB and MAPK activities in RAW 264.7 macrophages. *Evid Based Complement Alternat Med*. 2012;2012:637512.
29. Zhang F, Wang H, Wu Q, et al. Resveratrol protects cortical neurons against microglia-mediated neuroinflammation. *Phytother Res*. 2013;27(3):344–349.
30. Chen D, Nie M, Fan MW, Bian Z. Anti-inflammatory activity of curcumin in macrophages stimulated by lipopolysaccharides from *Porphyromonas gingivalis*. *Pharmacology*. 2008;82(4):264–269.
31. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. *Cold Spring Harb Perspect Biol*. 2009;1, a000034.
32. Kaltschmidt B, Kaltschmidt C. NF-kappaB in the nervous system. *Cold Spring Harb Perspect Biol*. 2009;1, a001271.
33. Scheinman RI, Cogswell PC, Lofquist AK, et al. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science*. 1995;270(5234):283–286.
34. Manna SK, Aggarwal BB. Immunosuppressive leflunomide metabolite (A77 1726) blocks TNF-dependent nuclear factor-kappa B activation and gene expression. *J Immunol*. 1999;162(4):2095–2102.
35. Min KS, Lee HJ, Kim SH, et al. Hydrogen peroxide induces heme oxygenase-1 and dentin sialophosphoprotein mRNA in human pulp cells. *J Endod*. 2008;34(8):983–989.