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Inhibitory effects of berberine on lipopolysaccharide-induced inducible nitric oxide synthase and the high-mobility group box 1 release in macrophages

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ABSTRACT

We investigated the molecular mechanism by which berberine reduces nitric oxide (NO) expression and high-mobility group box 1 (HMGB1) release in lipopolysaccharide (LPS)-induced macrophages. Berberine significantly inhibited the LPS-stimulated NO production and HMGB1 release in macrophages. In addition, berberine also induced heme oxygenase (HO)-1 in a dose-dependent manner, which was mediated through activation of p38 MAPK and NF-E2-related factor 2 (Nrf2) signaling cascade in macrophages. The inhibitory effect of berberine on LPS-stimulated NO and HMGB1 release was reversed by siRNA-Nrf2, SB203580 (p38 MAPK inhibitor) and zinc protoporphyrin (ZnPP; HO-1 inhibitor) within macrophages. Therefore, we conclude that berberine inhibits the proinflammatory response to LPS in macrophages by up-regulation of the HO-1 level, in which p38 MAPK and Nrf2 have an important role. These results suggest that berberine may be useful as a therapeutic agent for the treatment of inflammatory diseases.

1. Introduction

Severe sepsis and septic shock caused by invasive infection, represent systemic inflammation in response to invading pathogens [1]. The pathological sequence of sepsis is mediated by proinflammatory cytokines such as the tumor necrosis factor (TNF)-alpha, interleukin-1 (IL-1), and high-mobility group box 1 (HMGB1), that are released from innate immune cells [2]. Among proinflammatory cytokines, HMGB1 contributes to the high lethality of sepsis because of the late-acting downstream effectors. HMGB1 was first identified as a nuclear DNA binding protein and gene transcription and the activity of steroid hormone receptors [3–5]. Interestingly, administration of neutralizing antibodies to HMGB1 dramatically improved the survival of septic animals, thus suggesting it as a possibile therapeutic target for septic patients seen in an Emergency Department [6]. Recently, other studies have reported that HO-1 can be induced by stimulants such as cytokines, heat shock, heavy metals, and oxidants [7,8]. Interestingly, HO-1 can improve patient survival and decrease the circulating HMGB1 level in septic animals, which further suggests the beneficial effects of HO-1 for treating inflammatory disorders [9]. In oxidative injury and inflammation conditions, an increase in the synthesis of the HO-1 gene is linked to the transcription factor, NF-E2-related factor 2 (Nrf2)-ARE pathway [4,10]. It also suggests that Nrf2 nuclear translocation requires the activation of several signal transduction pathways, such as mitogen-activated protein kinases (MAPKs), Akt, and phosphatidylinositol 3-kinase (PI3K) [11]. It was recently reported that the HO-1 system provides a therapeutic effect in many experimental pathological conditions [12–14]. However, although effective therapeutic targets of inflammatory mediators may be ideal to treat sepsis, they have not yet been clinically approved.

Berberine, a natural isoquinoline alkaloid, has been used in Ayurvedic and Chinese Medicine for hundreds of years with a wide range of pharmacological and biochemical effects [15]. A growing number of studies have revealed that berberine has a wide variety of biological effects, including anti-tumor properties [16,17], cardiovascular-protective actions [18], antimicrobial effects [19], and antidiabetic properties [20]. More recently, berberine inhibited LPS-induced inflammation in microglia cells via regulation of HO-1 expression by modulating the Nrf2 signaling pathways [21]. Until now, the role of HMGB1 release and the related molecular mechanisms have not been completely clarified by berberine. Therefore, we demonstrate that berberine is able to induce HO-1 through

Abbreviations: iNOS, nitric oxide synthase; HMGB1, high-mobility group box 1; HO-1, heme oxygenase-1; ZnPP, zinc protopor-phyrin; LPS, lipopolysaccharide; Nrf2, NF-E2-related factor 2; TNF-α, tumor necrosis factor-alpha; MAPKs, mitogen-activated protein kinases; PI3K, phosphatidylinositol 3-kinase; ARE, antioxidant response element.

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activation of p38 MAPK and Nrf2 expression in macrophages and in turn to reduce HMGB1 under proinflammatory stimulus.

2. Materials and methods

2.1. Materials

Reagents used in this study were purchased from the following sources: berberine, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide], LPS and zinc protopor-phyrin (ZnPP) from Sigma–Aldrich (St. Louis, MO, USA); SB203580, PD98059, and SP600125 were purchased from Calbiochem (La Jolla, CA, USA); RPMI-1640, fetal bovine serum and Trizol were supplied by Gibco BRL (Grand Island, NY, USA); antibodies for p38, phospho-p38 MAPK, and HRP-conjugated anti-mouse IgG from Cell Signaling Technology (Beverly, MA); antibodies for HMGB1, iNOS, Nrf2 and HO-1 from Calbiochem (La Jolla, CA, USA). All chemicals and reagents were of analytical grade.

2.2. Animals

Specific pathogen-free BALB/C mice (female, 7 weeks old) were obtained from Central Laboratory Animal Inc. (Seoul, South Korea). Animals were housed under normal laboratory conditions, i.e. at 21–24 °C and 40–60% relative humidity under a 12 h light/dark cycle with free access to standard rodent food and water. All animals were raised under specific pathogen-free conditions, and the protocol was reviewed and approved by the Animal Subjects Committee of Asan Medical Center (Seoul, South Korea).

2.3. Preparation of peritoneal macrophages and cell culture

Peritoneal macrophages were isolated from mice that had been injected intraperitoneally with 2 ml of 4% (w/v) fluid thioglycollate medium 3 days prior to peritoneal lavage with 10 ml of RPMI 1640 medium. The collected cells were washed with RPMI 1640 and cultured in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin. Cells were plated on a flat-bottom culture plate and then incubated for 2 h at 37 °C in a 5% CO_2 humidified incubator.

2.4. Cell treatment and viability assay

Berberine was dissolved in dimethylsulfoxide (DMSO) and the stock solutions were added directly to the culture media. The control cells were treated with culture medium only. The final concentration of solvent was always <0.1%. The cells (5×10^3/well) in 10% FBS-RPMI-1640 were seeded into the 48-well plates. After incubation for 24 h, various concentrations of berberine and LPS were added to the well, and the plates were incubated at 37 °C for an additional 24 h. The cells were used for the MTT-based assay by measuring the according to the manufacturer's instructions. Relative cytotoxicity was quantified by absorption measurements at 550 nm using a microtiter plate reader (Molecular Devices, Menlo Park, CA, USA). This wavelength was not found to interfere with berberine.

2.5. NO assay

NO was measured as its stable oxidative metabolite, nitrite (NO_x), as previously described [22]. At the end of incubation, 100 μl of the culture medium was mixed with the same volume of Griess solution (0.1% naphthylethylenediamine and 1% sulfanilamide in 5% phosphoric acid). Light absorbance was measured at 550 nm using a microtiter plate reader (Molecular Devices, Menlo Park, CA, USA), and the nitrite concentration was determined using a curve calibrated on sodium nitrite standards.

2.6. HMGB1 release assay

The levels of HMGB1 in the culture medium were determined using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) and were performed according to the manufacturer's instructions.

2.7. RNA preparation and mRNA analysis by real-time quantitative PCR

The total RNA was isolated from cells using Trizol (GibcoBRL, Grand Island, NY, USA). Accumulated PCR products were directly detected by monitoring the increase in reporter dye (SYBR®). The expression levels of HO-1, iNOS and HMGB1 in the exposed cells were compared to those in the control cells at each time point using the comparative cycle threshold (*Ct*) method [24]. The following primer sequences were used: the HO-1 primers were sense 5′-CGCC TTCCTGCTCAACATT-3′ and antisense 5′-TGTGTTCCTCTGTCAGCATCAC-3′; the HMGB1 primers were sense 5′-TTGTGCAAACTTGCCGGGAGGA-3′ and antisense 5′-ACTTCTCCTTCAGCTTGGCAGC-3′; the iNOS primers were sense 5′-AACGGAGAACGTTGGATTTG and antisense 5′-CAGCACAAGGGGTTTTCTTC; Mouse ribosomal protein S18 (S18) sense 5′-GATGGGCGGGGGAAAAT-3′; and S18 antisense 5′-CTTGTACTGGCGTGGATTCTGC-3′. The quantity of each transcript was calculated as described in the instrument manual and was normalized to the amount of S18, a housekeeping gene.

2.8. Western immunoblot analysis

Cells were harvested and washed 3 times with cold phosphate-buffered saline (PBS). The cytoplasmic and nuclear protein fractions were extracted using NE-PER extraction reagents according to the manufacturer's protocol (Pierce Biotechnology, Rockford, IL). Cytoplasmic/nuclear protein extracts or whole protein extracts were used for Western blot analysis. Western blotting was performed using anti-HO-1, anti-iNOS, anti-HMGB1, anti-p38, anti-phospho-p38, and anti-actin antibodies. Protein samples were heated at 95 °C for 5 min and were analyzed using SDS–PAGE. Immunoblot signals were developed by enhanced chemiluminescence (Pierce Biotechnology, Rockford, IL, USA).

2.9. siRNA knockdown

siNrf2 and scrambled siRNA were acquired from Santa Cruz Biotechnology. siRNA was transfected into macrophages according to the manufacturer's protocol and using the transfection reagent, Lipofectamine 2000© (Invitrogen, CA, USA). The cells were incubated with 100 nM of target siRNA or scramble siRNA for 4 h in serum- and antibiotic-free media. The cells were then incubated for 18°h in media containing antibiotics and FBS, and cells were washed, pretreated with or without berberine for 1 h, and treated with LPS.

2.10. Transient transfection and luciferase assay

Cells (3×10^5 cells/well) were seeded in 24-well plates, incubated overnight and transiently co-transfected with ARE-promoter-luciferase construct and pRL-SV40 plasmid (*Renilla* luciferase expression for normalization) (Promega, Madison, WI, USA) using LipofectAMINE™ 2000 reagent (Invitrogen, Carlsbad, CA, USA). Relative luciferase activities were calculated by normalizing ARE-promoter-driven firefly luciferase activity to *Renilla* luciferase activity.

2.11. Statistical analysis

All the experiments were repeated at least three times. The results are expressed as a mean ± SD, and the data were analyzed using one-way ANOVA followed by a Student's *t* test for significant difference. A p value <0.05 was considered significant.

3. Results

3.1. Berberine inhibits inflammation-related genes and proteins expression

The cytotoxicity of berberine was assessed by MTT assay which demonstrated that the concentration of berberine used in these experiments did not decrease the cell viability (>95% cell viability, Fig. 1A). To identify the potential anti-inflammatory effect of berberine, macrophages were incubated with berberine in the presence of LPS or alone. As shown in Fig. 1A and B, berberine inhibited production of NO production as well as HMGB1 release in LPS-activated macrophages in does dependent manner. Moreover, berberine significantly inhibited production of inducible nitric oxide synthase (iNOS) protein and gene expression (Fig. 1C), as well as HMGB1 (Fig. 1D) in LPS-activated macrophages. Therefore, we confirmed the previous report that berberine can inhibit the expression of pro-inflammatory protein such as iNOS [25] and it is the first report that berberine inhibits HMGB1 releases in LPS-induced macrophage cells.

3.2. Berberine induces HO-1 expression through p38 MAPK, but not ERK or JNK

We then investigated whether berberine can induce HO-1 in macrophages. After cells were treated with berberine for 24 h, the treatment dose-dependently increased both HO-1 mRNA and protein expression (Fig 2A). This increase was sensitive to pretreatment with actinomycin D, thus suggesting that berberine enhances the expression of the inducible heme oxygenase isoform, HO-1. Moreover, the increase in mRNA was also sensitive to cycloheximide, and suggesting that induction of HO-1 transcription involves de novo protein synthesis (Fig 2B). To identify which MAPK signaling pathway involved in up-regulating of HO-1 by berberine, we used different pharmacological signal inhibitors. First, we tested that signal inhibitors by themselves (SB203580, p38 inhibitor; SP600125, JNK inhibitor; and PD98059, ERK inhibitor) did not affect HO-1 expression in macrophages (Data not shown) and, we found that berberine then significantly induced HO-1 in macrophage, which was inhibited only by SB203580 but not by either SP600125 or PD98059 (Fig. 2C). Consistent with previous data, berberine significantly induced p38 activation in macrophages in does dependent manner (Fig. 2D). Overall, we suggest that p38 has a key role in berberine-mediated HO-1 induction.

3.3. Berberine activates Nrf-2 in LPS-activated macrophages

Among the transcription factors, Nrf2 is important as it regulates ARE-driven, HO-1 gene expression [4,26]. To understand whether p38 MAPK has a key role for inducting HO-1 via Nrf2 activation, we investigated the Nrf2 translocation and ARE-luciferase activity caused by berberine and found that berberine stimulated nuclear accumulation of Nrf2 in macrophages (Fig. 3A). Consistent with previous studies, berberine increased ARE-luciferase activity, which was significantly reduced by SB203580 but not by either SP600125 or PD98059, thus indicating that the Nrf2 activation by berberine is dependent on p38 activity (Fig. 3B). To further determine whether berberine-mediated HO-1 expression mediated by Nrf2 activation, we used siNrf2. As shown in Fig. 3C, berberine significantly reduced HO-1 expression in siNrf2 transfected cells suggesting the critical role of Nrf2 signaling in HO-1 expression. In addition, SB203580 significantly reduced Nrf2 expression and HO-1 expression by berberine (Fig. 3C).

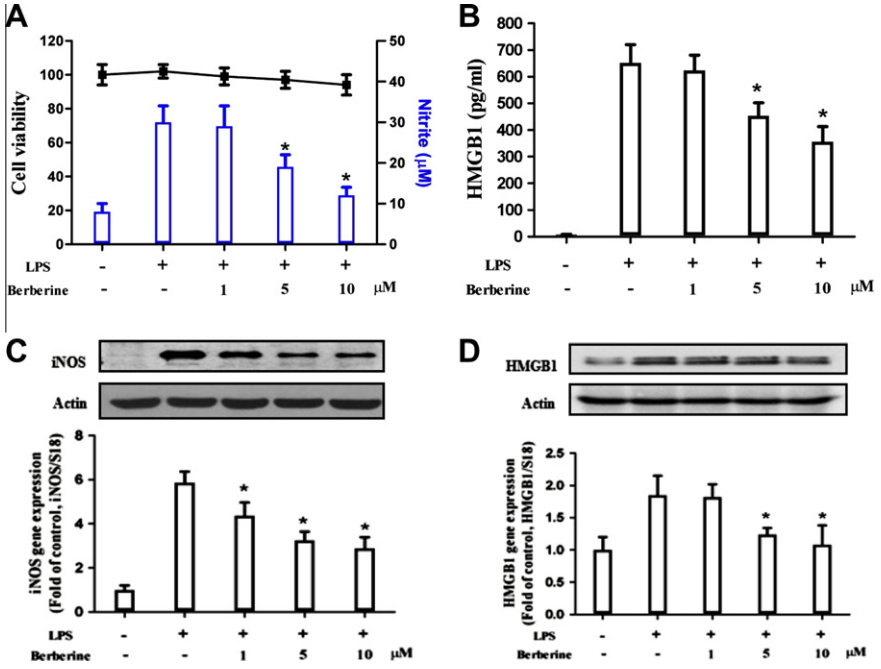

Fig. 1. Effect of berberine on the expression of NO production and HMGB1 release in LPS-stimulated macrophages. Cells were pretreated with berberine for 1 h at doses 1, 5, and 10 μM. The cells were then stimulated with LPS (1 μg/ml) for 24 h. Cell viability was measured by MTT assays (A). The culture medium was collected and subjected to NO production analysis (A) and Western blot and release for HMGB1 analysis (B and D). Cells were lysed, harvested and subjected to western blot and RT-PCR for iNOS (C) and RT-PCR for HMGB1 (D) detection as described in Section 2. *Significantly different from berberine plus LPS-treated cells.

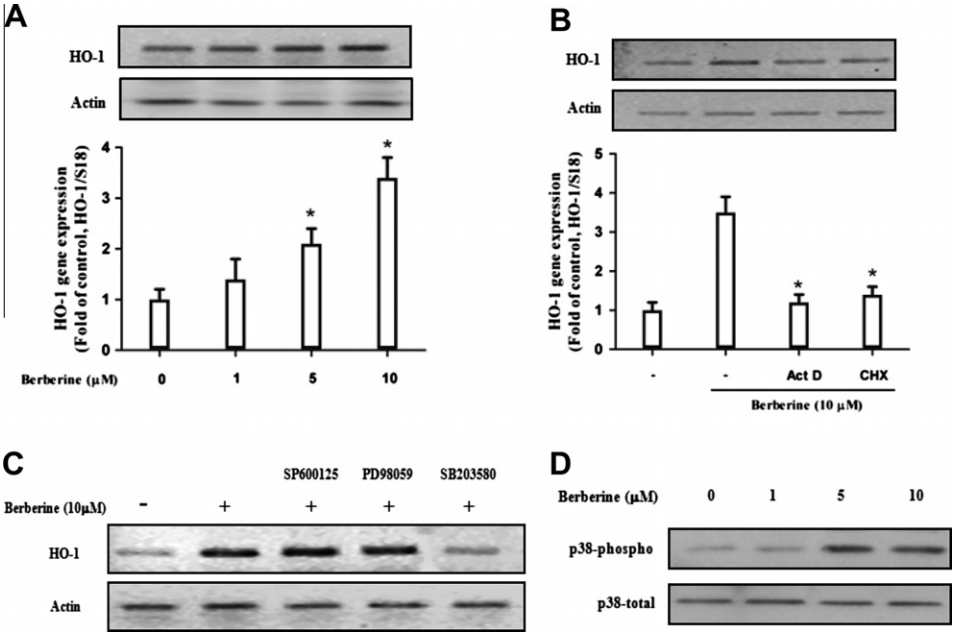

Fig. 2. Effect of berbrein on the expression of HO-1 through p38 MAPK in macrophages. Cells were exposed to various concentrations of berbrein for 24 h and total RNA and protein were extracted. HO-1 mRNA and protein expression were analyzed by RT-PCR and Western blotting (A). Cells were untreated or pretreated with 50 μM cycloheximide or 10 μg/ml actinomycin D for 2 h prior to the addition of 10 μM berbrein for an additional 24 h (B). *Significantly different from berberine-treated cells. Cells were pretreated with SB203580 (10 μM), SP600125 (10 μM), and PD98059 (10 μM) for 1 h, and then cells were treated with berberine (10 μM) for 24 h. After incubation, cells were harvested and subjected to western blot as described in Section 2 (C). Cells were exposed to various concentrations of berbrein for 3 h and protein was extracted. Parallel immunoblots were analyzed for total kinase levels with anti-p38 antibodies (D).

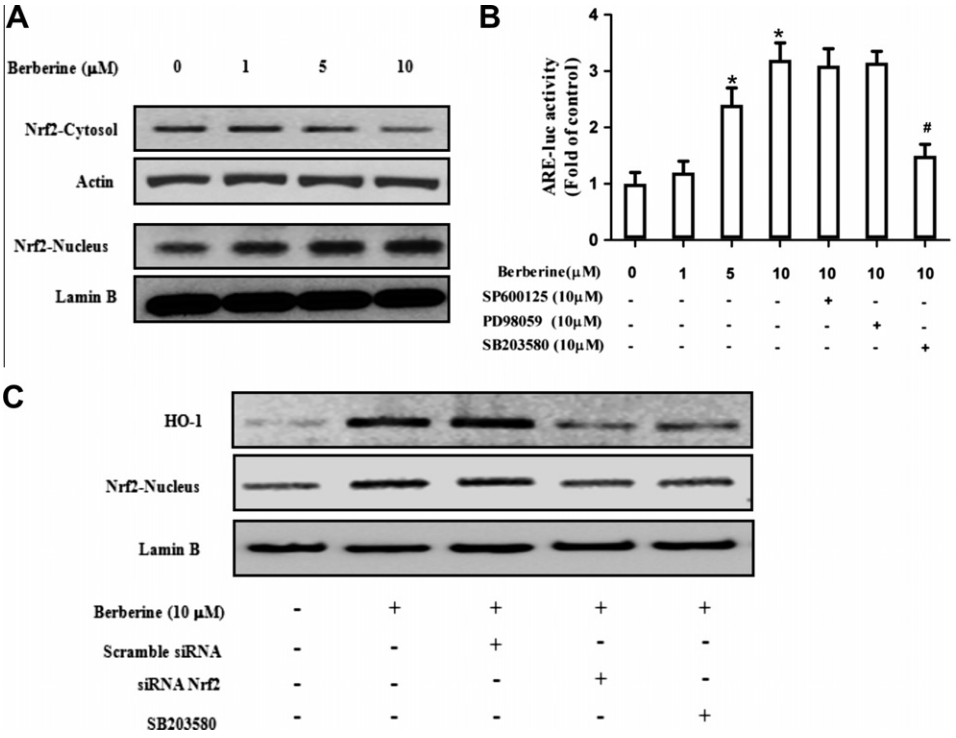

Fig. 3. Effect of berberine on Nrf2 translocation into nucleus and its transcriptional activity. macrophages were treated with berberine (10 μM) for the indicated time and then nuclear and cytosol extracts were prepared for Western blotting. Cell extracts were subjected to Western blot for HO-1 expression (A). Cells were pretreated with SB203580 (10 μM), SP600125 (10 μM), and PD98059 (10 μM) for 1 h, and then cells were treated with berberine (10 μM) for 24 h. After incubation, cells were subjected to luciferase assay as described in Section 2 (B). Cells were transfected with scramble (siRNA) or siNrf2 as described in Section 2. After 24 h incubation with berberine (10 μM) or bereberine + inhibitors, cells were harvested and subjected to Western blot for HO-1 expression (C). Transfection efficiency was confirmed by checking Nrf2 expression. *Significantly different from berberine-treated cells. #Significantly different from berberine plus LPS-treated cells.

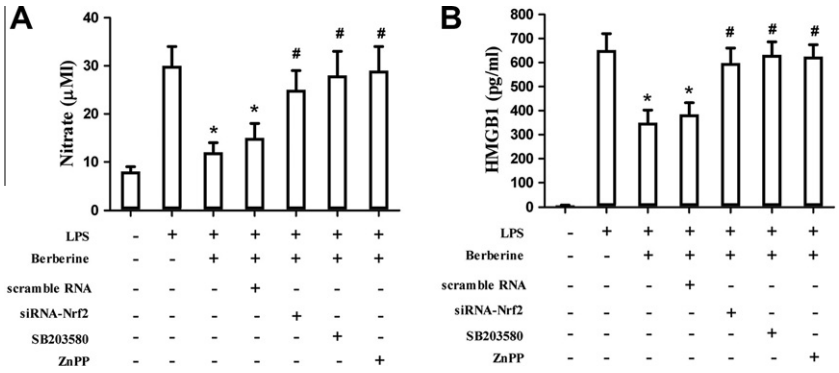

Fig. 4. Berberine regulates anti-inflammatory action by HO-1 down-regulation through p38, Nrf2 pathways in macrophages. Cells were transfected with Nrf2-siRNA or Scramble siRNA and cells were incubated with berberine (10 μM) with or without SB203580 (10 μM) for 1 h and stimulated with LPS (1 μg/ml) for 24 h. Then, culture medium samples were subjected to assay for NO production (A) and HMGB1 release (B), respectively. *Significantly different from LPS-treated cells and #Significantly different from berberine plus LPS-treated cells.

3.4. Anti-inflammatory effect of berberine is mediated through HO-1

As berberine has potent anti-inflammatory activities [27], we investigated why HO-1 induction is important for berberine-mediated anti-inflammation. We fist checked the efficiency of ZnPP (HO-1inhibitor), SB203580 and siRNA-Nrf2 of berberine-induced NO and HMGB1 release. Our results showed that berberine-induced HO-1 expression-mediated inhibition of NO production (Fig. 4A) and HMGB1 release (Fig. 4B) was also reversed in the presence SB203580, siRNA-Nrf2 and ZnPP, and thus suggesting the importance of p38/HO-1 signaling as part of the anti-inflammatory properties of berberine-induced HO-1 expression.

4. Discussion

Environmental chemicals can change immune function by regulating cytokines. In vitro studies using established immune cell lines have been useful for describing the effects of chemicals on immune function. A previous report showed that berberine effectively inhibits LPS- and IFN-γ-induced neuro-inflammation in microglia cells [28]. In fact, berberine induces heme oxygenase-1 expression through phosphatidylinositol 3-kinase/AKT and the NF-E2-related factor-2 signaling pathway in astrocytes [25]. However, the precise anti-sepsis mechanisms in macrophages by berberine remain unclear. In the present study, we showed direct evidence that berberine contributed to the down-regulation of HMGB1 and iNOS (NO) by HO-1 induction through activation of p38 MAPK and Nrf2 in macrophages. Numerous studies have suggested that HMGB1 mediates cognitive impairment in sepsis survivors [2,29]. Moreover, previous studies have showed that HO-1 inhibits the release of HMGB1 in RAW264.7 cells activated by LPS and in LPS- or cecal ligation and puncture -induced septic mice in vivo [8]. These results suggest that reduction of HMGB1 and iNOS by HO-1 induction might serve as an important mechanism for achieving the anti-sepsis effects of berberine.

The questing then becomes what is the signal mechanism by which berberine induces HO-1? Because activation of MAPKs has a central role in the induction of HO-1 [30] and iNOS [31,32] gene expression, it is of use to investigate which MAPKs are responsible for berberine-mediated HO-1 and iNOS induction. These findings indicate the complexity of the role of p38 MAPK in the regulation of iNOS in macrophages, and under inflammatory stimuli. We found that p38 MAPK, although neither JNK nor ERK, significantly mediated berberine-induced HO-1 induction, and thus indicating that p38 MAPK has a crucial role in HO-1 induction. Therefore, it has been shown that the byproducts of HO-1 provide an anti-inflammatory effect through the activation of p38 MAPK. Because both the induction of HO-1 and the anti-inflammatory effect of berberine were reversed by the presence of SB203580, we suggest that p38 has a key role in berberine-mediated HO-1 induction.

The HO-1 gene promoter contains multiple regulatory transcription factor binding sites, including antioxidant response element (ARE), NF-κB, AP-1, and AP-2 responsive elements [10,19,33]. The transcription factor, Nrf2, positively regulates the ARE-mediated expression of phase II detoxification enzyme genes, including HO-1 [34,35]. Ours is the first report demonstrating that berberine consistently increases the expression of HO-1 genes through Nrf2-mediated ARE activation. Moreover, p38 inhibitor blocked berberine-induced Nrf2 nuclear translocation. These results suggest that p38 pathways are important for berberine-induced HO-1 expression and for Nrf2 nuclear translocation.

In summary, berberine induces HO-1 expression in macrophage cells, and this expression induces anti-inflammation against sepsis and also induces Nrf2 nuclear translocation, which is upstream of berberine-induced HO-1 expression, and activates p38 phosphorylation. These results suggest that berberine may be useful as a therapeutic agent for the treatment of inflammatory diseases such as sepsis.

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References

[1] M. Aziz, A. Jacob, W.L. Yang, A. Matsuda, P. Wang, Current trends in inflammatory and immunomodulatory mediators in sepsis, J. Leukoc. Biol. (2012).
[2] W. Huang, Y. Tang, L. Li, HMGB1, a potent proinflammatory cytokine in sepsis, Cytokine 51 (2010) 119–126.
[3] J.O. Thomas, K. Stott, H1 and HMGB1: modulators of chromatin structure, Biochem. Soc. Trans. 40 (2012) 341–346.
[4] K. Srisook, C. Kim, Y.N. Cha, Molecular mechanisms involved in enhancing HO-1 expression: de-repression by heme and activation by Nrf2, the "one-two" punch, Antioxid. Redox Signal. 7 (2005) 1674–1687.
[5] M.T. Lotze, K.J. Tracey, High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal, Nat. Rev. Immunol. 5 (2005) 331–342.
[6] A.E. Sama, J. D'Amore, M.F. Ward, G. Chen, H. Wang, Bench to bedside: HMGB1-a novel proinflammatory cytokine and potential therapeutic target for septic patients in the emergency department, Acad. Emerg. Med. 11 (2004) 867–873.
[7] H.J. Jang, Y.M. Kim, K. Tsoyi, E.J. Park, Y.S. Lee, H.J. Kim, J.H. Lee, Y. Joe, H.T. Chung, K.C. Chang, Ethyl pyruvate induces heme oxygenase-1 through p38 mitogen-activated protein kinase activation by depletion of glutathione in RAW 264.7 cells and improves survival in septic animals, Antioxid. Redox Signal. 17 (2012) 878–889.

[8] K. Tsoyi, T.Y. Lee, Y.S. Lee, H.J. Kim, H.G. Seo, J.H. Lee, K.C. Chang, Heme-oxygenase-1 induction and carbon monoxide-releasing molecule inhibit lipopolysaccharide (LPS)-induced high-mobility group box 1 release in vitro and improve survival of mice in LPS- and cecal ligation and puncture-induced sepsis model in vivo, Mol. Pharm. 76 (2009) 173–182.
[9] M.L. Wu, Y.C. Ho, C.Y. Lin, S.F. Yet, Heme oxygenase-1 in inflammation and cardiovascular disease, Am. J. Cardiovasc. Dis. 1 (2011) 150–158.
[10] Y.J. Surh, J.K. Kundu, M.H. Li, H.K. Na, Y.N. Cha, Role of Nrf2-mediated heme oxygenase-1 upregulation in adaptive survival response to nitrosative stress, Arch. Pharm. Res. 32 (2009) 1163–1176.
[11] D. Martin, A.I. Rojo, M. Salinas, R. Diaz, G. Gallardo, J. Alam, C.M. De Galarreta, A. Cuadrado, Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol, J. Biol. Chem. 279 (2004) 8919–8929.
[12] E.O. Farombi, Y.J. Surh, Heme oxygenase-1 as a potential therapeutic target for hepatoprotection, J. Biochem. Mol. Biol. 39 (2006) 479–491.
[13] N.F. Luz, B.B. Andrade, D.F. Feijo, T. Araujo-Santos, G.Q. Carvalho, D. Andrade, D.R. Abanades, E.V. Melo, A.M. Silva, C.I. Brodskyn, M. Barral-Netto, A. Barral, R.P. Soares, R.P. Almeida, M.T. Bozza, V.M. Borges, Heme oxygenase-1 promotes the persistence of Leishmania chagasi infection, J. Immunol. 188 (2012) 4460–4467.
[14] M. Bonelli, A. Savitskaya, C.W. Steiner, E. Rath, M. Bilban, O. Wagner, F.H. Bach, J.S. Smolen, C. Scheinecker, Heme oxygenase-1 end-products carbon monoxide and biliverdin ameliorate murine collagen induced arthritis, Clin. Exp. Rheumatol. 30 (2012) 73–78.
[15] M. Imanshahidi, H. Hosseinzadeh, Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine, Phytother. Res. 22 (2008) 999–1012.
[16] N. Tong, J. Zhang, Y. Chen, Z. Li, Y. Luo, H. Zuo, X. Zhao, Berberine sensitizes mutliple human cancer cells to the anticancer effects of doxorubicin in vitro, Oncol. Lett. 3 (2012) 1263–1267.
[17] W. Tan, Y. Li, M. Chen, Y. Wang, Berberine hydrochloride: anticancer activity and nanoparticulate delivery system, Int. J. Nanomedicine 6 (2011) 1773–1777.
[18] C.W. Lau, X.Q. Yao, Z.Y. Chen, W.H. Ko, Y. Huang, Cardiovascular actions of berberine, Cardiovasc. Drug Rev. 19 (2001) 234–244.
[19] J.M. Boberek, J. Stach, L. Good, Genetic evidence for inhibition of bacterial division protein FtsZ by berberine, PLoS ONE 5 (2010) e13745.
[20] J. Yin, H. Zhang, J. Ye, Traditional chinese medicine in treatment of metabolic syndrome, Endocr. Metab. Immune Disord. Drug Targets 8 (2008) 99–111.
[21] Y.Y. Hsu, C.S. Chen, S.N. Wu, Y.J. Jong, Y.C. Lo, Berberine activates Nrf2 nuclear translocation and protects against oxidative damage via a phosphatidylinositol 3-kinase/Akt-dependent mechanism in NSC34 motor neuron-like cells, Eur. J. Pharm. Sci. 46 (2012) 415–425.
[22] J.Y. Kim, K.S. Jung, K.J. Lee, H.K. Na, H.K. Chun, Y.H. Kho, H.G. Jeong, The coffee diterpene kahweol suppress the inducible nitric oxide synthase expression in macrophages, Cancer Lett. 213 (2004) 147–154.
[24] M.R. Johnson, K. Wang, J.B. Smith, M.J. Heslin, R.B. Diasio, Quantitation of dihydropyrimidine dehydrogenase expression by real-time reverse transcription polymerase chain reaction, Anal. Biochem. 278 (2000) 175–184.
[25] J.H. Chen, S.M. Huang, T.W. Tan, H.Y. Lin, P.Y. Chen, W.L. Yeh, S.C. Chou, C.F. Tsai, I.H. Wei, D.Y. Lu, Berberine induces heme oxygenase-1 up-regulation through phosphatidylinositol 3-kinase/AKT and NF-E2-related factor-2 signaling pathway in astrocytes, Int. Immunopharmacol. 12 (2012) 94–100.
[26] J. Alam, J.L. Cook, Transcriptional regulation of the heme oxygenase-1 gene via the stress response element pathway, Curr. Pharm. Des. 9 (2003) 2499–2511.
[27] S. Manoharan, G. Sindhu, M.R. Nirmal, V. Vetrichelvi, S. Balakrishnan, Protective effect of berberine on expression pattern of apoptotic, cell proliferative, inflammatory and angiogenic markers during 7,12-dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis, Pak. J. Biol. Sci. 14 (2011) 918–932.
[28] D.Y. Lu, C.H. Tang, Y.H. Chen, I.H. Wei, Berberine suppresses neuroinflammatory responses through AMP-activated protein kinase activation in BV-2 microglia, J. Cell. Biochem. 110 (2010) 697–705.
[29] S.S. Chavan, P.T. Huerta, S. Robbiati, S.I. Valdes-Ferrer, M. Ochani, M. Dancho, M. Frankfurt, B.T. Volpe, K.J. Tracey, B. Diamond, Response to "HMGB1 mediates cognitive impairment in sepsis survivors", Mol. Med. 18 (2012) 1359.
[30] J. Alam, J.L. Cook, How many transcription factors does it take to turn on the heme oxygenase-1 gene?, Am J. Respir. Cell Mol. Biol. 36 (2007) 166–174.
[31] Q.W. Xie, Y. Kashiwabara, C. Nathan, Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase, J. Biol. Chem. 269 (1994) 4705–4708.
[32] A. Lahti, O. Sareila, H. Kankaanranta, E. Moilanen, Inhibition of p38 mitogen-activated protein kinase enhances c-Jun N-terminal kinase activity: implication in inducible nitric oxide synthase expression, BMC Pharmacol. 6 (2006) 5.
[33] Y. Lavrovsky, M.L. Schwartzman, R.D. Levere, A. Kappas, N.G. Abraham, Identification of binding sites for transcription factors NF-kappa B and AP-2 in the promoter region of the human heme oxygenase 1 gene, Proc. Natl. Acad. Sci. USA 91 (1994) 5987–5991.
[34] X. Zhang, L. Lu, C. Dixon, W. Wilmer, H. Song, X. Chen, B.H. Rovin, Stress protein activation by the cyclopentenone prostaglandin 15-deoxy-delta12,14-prostaglandin J2 in human mesangial cells, Kidney Int. 65 (2004) 798–810.
[35] Y.P. Hwang, H.G. Jeong, Ginsenoside Rb1 protects against 6-hydroxydopamine-induced oxidative stress by increasing heme oxygenase-1 expression through an estrogen receptor-related PI3K/Akt/Nrf2-dependent pathway in human dopaminergic cells, Toxicol. Appl. Pharmacol. 242 (2010) 18–28.