Emodin Inhibits Proinflammatory Responses and Inactivates Histone Deacetylase 1 in Hypoxic Rheumatoid Synoviocytes

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Chronic inflammation of rheumatoid arthritis (RA) is promoted by proinflammatory cytokines and closely linked to angiogenesis. In the present study, we investigated the anti-inflammatory effects of emodin (1,3,8-trihydroxy-6-methyl-anthraquinone) isolated from the root of *Rheum palmatum* L. in interleukin 1 beta (IL-1β) and lipopolysaccharide (LPS)-stimulated RA synoviocytes under hypoxia. Emodin significantly inhibited IL-1β and LPS-stimulated proliferation of RA synoviocytes in a dose-dependent manner under hypoxic condition. Also, enzyme linked immunosorbent assay (ELISA) revealed that emodin significantly reduced the production of proinflammatory cytokines [tumor necrosis factor-alpha (TNF-α), IL-6 and IL-8], mediators [prostagladin E_2 (PGE_2), matrix metalloproteinase (MMP)-1 and MMP-13] and vascular endothelial growth factor (VEGF) as an angiogenesis biomarker in IL-1β and LPS-treated synoviocytes under hypoxia. Consistently, emodin attenuated the expression of cyclooxygenase 2 (COX-2), VEGF, hypoxia inducible factor 1 alpha (HIF-1α), MMP-1 and MMP-13 at mRNA level in IL-1β and LPS-treated synoviocytes under hypoxia. Furthermore, emodin reduced histone deacetylase (HDAC) activity as well as suppressed the expression of HDAC1, but not HDAC2 in IL-1β and LPS-treated synoviocytes under hypoxia. Overall, these findings suggest that emodin inhibits proinflammatory cytokines and VEGF productions, and HDAC1 activity in hypoxic RA synoviocytes.

Key words emodin; synoviocyte; inflammation; vascular endothelial growth factor; histone deacetylase 1

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease that primarily attacks synovial joints, and other tissues and organs. One of RA features is the expansion of fibroblast-like synoviocytes.[1] Excessive production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6 and IL-8 mediates abnormal proliferation of synoviocytes that results in pathological changes such as joint ankylosis and the destruction of articular cartilage in an RA joint.[1]

Hypoxia, a pathological condition with deficient oxygen supply,[2] mainly activates the transcription factor hypoxia inducible factor 1 (HIF-1) and consequently promotes the release of vascular endothelial growth factor (VEGF).[3] Many papers reported that the importance of VEGF in angiogenesis is correlated with inflammatory diseases including RA.[4—6] VEGF level is significantly high in RA synovial fluid.[7] Blocking VEGF using VEGF inhibitors[8,9] or small interfering RNA (siRNA) for VEGF receptor 2 (VEGFR2) suppressed secretion of proinflammatory cytokines that indirectly contribute to inhibit angiogenesis.[10] In addition, angiogenesis promoted by VEGF stimulates the release of proinflammatory mediators such as cyclooxygenase 2 (COX-2),[8] matrix metalloproteinases (MMPs)[11] and inducible nitric oxide synthase (iNOS).[12] Recently, TNF-α-stimulated synoviocytes increase histone deacetylase (HDAC) activity and expression, implying that HDAC is another important transcription factor to regulate inflammatory response of synoviocytes.[13]

Emodin (1,3,8-trihydroxy-6-methyl-anthraquinone) (Fig. 1A) is a natural anthraquinone compound isolated from the root and rhizomes of *Rheum palmatum* L. with various biomedical properties such as anti-cancer,[14] anti-viral,[15] anti-oxidant[16] and anti-inflammatory activities.[17,18] Nonetheless, the underlying mechanisms for its anti-inflammatory effects still remain unclear. Thus, in the present study, we investigated whether or not emodin exerts anti-inflammatory property in IL-1β and lipopolysaccharide (LPS)-stimulated synoviocytes under hypoxic condition using proliferation assay, enzyme-linked immunosorbent assay (ELISA), reverse transcription-polymerase chain reaction (RT-PCR) and Western

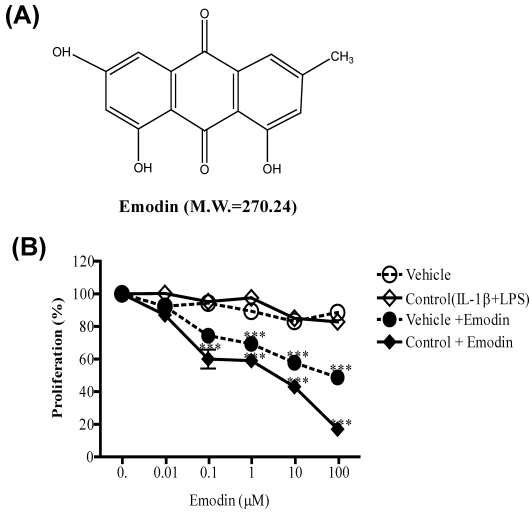

Fig. 1. Effect of Emodin on the Proliferation of IL-1β and LPS-Stimulated Synoviocytes

(A) Chemical structure of emodin. Molecular weight=270.24. (B) Cells were stimulated with IL-1β and LPS, and exposed to various concentrations of emodin (0, 0.01, 0.1, 1, 10 or 100 μM) for 72 h under hypoxia. Cell proliferation was determined by BrdU assay. Data shown are representative from three independent experiments. Values are expressed as means±standard error (S.E.). *** $p<0.001$ *vs.* untreated control.

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blotting.

MATAERIALS AND METHODS

Cell Culture and Hypoxia Induction Synovial membranes were obtained from three patients with rheumatoid arthritis (17, 28, and 60-years-old) at the time of surgery for total knee replacement. The samples obtained from synovial regions were verified based on a pathologic diagnosis under IRB approval of Kyung Hee Medical Center following appropriate written consent for the use of human ovarian tissues. Synoviocytes were prepared by 0.2% type II collagenase digestion method as described.[19] Briefly, synovial membranes were washed with phosphate buffered saline (PBS) and finely minced. Cells were released by treating synovial membranes for 2 h with 0.2% type II collagenase (Sigma-Aldrich Co., MO, U.S.A.) at 37 °C in high-glucose Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Corp., Carlsbad, CA, U.S.A.) supplemented with 10% fetal bovine serum (FBS) and antibiotic–antimycotic. After removing undigested tissues using a 70-μm nylon sieve, cells were collected by centrifugation, suspended with high-glucose DMEM supplemented with 10% FBS and antibiotics, plated and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 3 d to allow cells to adhere and grow to confluence, and split at a 1 : 4 ratio. Synoviocytes (passages 3 to 6) were used for all experiments.

For hypoxia induction, culture medium was replaced to a thin layer of fresh serum-free medium to decrease the diffusion distance of the ambient gas. The cells were incubated in a Bactron Anaerobic/Environmental Chamber (Sheldon Manufacturing, Inc.) with 1% O_2, 5% CO_2, and 95% N_2 at 37 °C.

Cell Proliferation Assay Cell proliferation studies were performed by 5-bromo-2′-deoxyuridine (BrdU) colorimetric assay as previously described.[20] Cells were plated at 2×10^4 cells/well onto 96-well plate and treated with various concentrations of emodin (0, 0.01, 0.1, 1, 10 or 100 μM) in the presence or absence of IL-1β and LPS for 72 h. The samples were added with BrdU for additional 6 h, fixed with FixDenat solution for 30 min and incubated with anti-BrdU followed by the horseradish peroxidase reaction. The reaction was terminated by adding 1 M H_2SO_4, and the absorbance was quantified by ELISA reader at 480—650 mm.

Enzyme-Linked Immunosorbent Assay (ELISA) ELISA was performed by using R&D systems ELISA kit (Minneapolis, MN, U.S.A.). Synovial cells (2.5×10^5 cells per 60-mm dish per 2 ml of serum-free media) were treated with recombinant IL-1β (10 ng/ml) (R&D Systems, Inc., Minneapolis, MN, U.S.A.) and LPS (100 ng/ml) (Sigma-Aldrich, St. Louis, MO, U.S.A.) and/or emodin (0, 1, or 10 μM) under normoxia or hypoxia for 24 h. Culture supernatants were collected and subjected ELISA for IL-6, IL-8, TNF-α, VEGF, MMP-1, and MMP-13. Three independent experiments were performed in quadruplicates. Each experiment was performed using synovial cells isolated from RA patients.

Reverse-Transcriptase Polymerase Chain Reaction Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions as previously described.[21] cDNA synthesis and PCR reaction were carried out using the system for RT-PCR and TaKaRa Taq™ (TaKaRa Biotechnology, Shiga, Japan). PCR primer sequences (Bioneer, Deajeon, Korea) are listed in Table 1.

Table 1. Primer Sequences for RT-PCR Analysis

mRNA	Primer sequences	Product size
MMP-1	F: 5′-CCTAGCTACACCTTCAGTGG-3′ R: 5′-GCCCAGTACTTATTCCCTTT-3′	338 bp
MMP-13	F: 5′-TTGAGGATACAGGCAAGACT-3′ R: 5′-TGGAAGTATTACCCCAAATG-3′	311 bp
COX-2	F: 5′-TTCAAATGAGATTGTGGGAAAATTGCT-3′ R: 5′-AGATCATCTCTGCCTGAGTATCTT-3′	306 bp
VEGF	F: 5′-TCTTGGGTGCATTGGAGCCTC-3′ R: 5′-AGCTCATCTCTCCTATGTGC-3′	426 bp
β-Actin	F: 5′-GCTCTCCAGAACATCACTCCTGCC-3′ R: 5′-CGTTGTCATACCAGGAAATGAGCT-3′	250 bp

F, forward; R, reverse; bp, base pairs; MMP, matrix metalloproteinase; COX-2, cyclooxygenase 2; VEGF, vascular endothelial growth factor.

Western Blot Analysis Western blotting was performed as previously described.[22] Synoviocytes were serum-starved overnight and stimulated with recombinant IL-1β (10 ng/ml) and LPS (100 ng/ml) and/or emodin (10 μM) for 24 h under hypoxia. Cells were lysed in lysis buffer [20 mM Tris–Cl, pH 8.0, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, 20 μg/ml chymostatin, 2 mM phenylmethylsulphonyl fluoride (PMSF), 10 μM leupeptin, and 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF)]. The protein samples were separated onto 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Hybond-enhanced chemiluminescence (ECL) membranes (GE Healthcare, Buckinghamshire, U.K.). The membranes were blocked in 6% nonfat milk dissolved in TBST buffer (10 mM Tris–Cl, pH 8.0, 150 mM NaCl and 0.05% Tween 20) and probed with primary antibodies for *p*-extracellular signal-regulated kinase (ERK), ERK, p-p38, p38, HDAC1, HDAC2 (Cell Signaling, Danvers, MA, U.S.A.) and β-actin (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) at 4 °C overnight and goat anti-rabbit immunoglobulin G (IgG) secondary antibody coupled with horseradish peroxidase at room temperature for 2 h. The blots were developed using the ECL system (GE Healthcare, Buckinghamshire, U.K.). For re-probing, the blots were incubated in the stripping buffer (100 mM 2-mercaptoethanol, 2% SDS and 62.5 mM Tris–HCl, pH 6.7) at 50 °C for 30 min.

HDAC Activity Assay HDAC activity was analyzed by using HDAC assay kit (Millipore, Temecula, CA, U.S.A.) following manufacturer's instructions. Cell lysates (20 μg of nuclear protein) were incubated with HDAC assay substrate for 60 min at 37 °C. Activator solution was then added, and the mixture was incubated for 15 min at room temperature. Optical density (OD) values were measured using a fluorescence microplate reader at 405 nm.

Electrophoretic Mobility Shift Assay (EMSA) The nuclear factor kappa B (NF-κB)-DNA binding was analyzed by electrophoretic mobility shift assay (EMSA) using Gelshift Chemiluminescent EMSA kit (Active Motif, Carlsbad, CA, U.S.A.). Briefly, nuclear extracts were prepared and incubated with NF-κB consensus oligonucleotides (5′-AGT TGA GGG GAC TTT CCC AGG C-3′) (Santa Cruz Biotechnologies, Santa Cruz, CA, U.S.A.). The DNA–protein complex

formed was separated from free oligonucleotides on 5% native polyacrylamide gels. Chemiluminescent detection was performed using ECL reagents according to the vendor's protocols (GE Health Care Bio-Sciences, Piscataway, NJ, U.S.A.).

Statistical Analyses Data are expressed as means±standard error (S.E) from quadruplicate samples. The statistically significant differences were calculated by Student's *t*-test or Dunnett multiple comparison test using Prism software 4 (GraphPad Software, Inc., San Diego, CA, U.S.A.). p value less than 0.05 was considered as statistically significant.

RESULTS

Emodin Inhibited the Proliferation of IL-1β and LPS-Stimulated Synoviocytes Hypoxia is a major feature of the microenvironment in the synovium of RA, a chronic inflammation of the joints.[23] Cell proliferation was measured to evaluate effect of emodin on the proliferation of synoviocytes with or without IL-1β and LPS under hypoxia. As shown in Fig. 1B, emodin significantly inhibited the cell proliferation in a dose-dependent manner under hypoxia. Interestingly, in the presence of IL-1β and LPS, the inhibitory effect of emodin was accentuated on the proliferation of synoviocytes. In contrast, the treatment of IL-1β and LPS did not show any significant effect on the proliferation of synoviocytes compared to untreated control.

Emodin Inhibited Proinflammatory Cytokine Production in IL-1β and LPS-Stimulated Synoviocytes To examine effects of emodin on the inflammatory responses in synoviocytes, the cells were treated with emodin in the presence of IL-1β and LPS under hypoxic condition. Levels of cytokines TNF-α, IL-6 and IL-8 were measured by ELISA. Whilst hypoxia itself had no influence on the cytokine production, the stimulation of IL-1β and LPS dramatically increased the levels of TNF-α (A), IL-6 (B) and IL-8 (C) under hypoxia (Fig. 2, lane 3). Of note, emodin significantly inhibited the secreted levels of TNF-α (A), IL-6 (B) and IL-8 (C) in a dose-dependent manner under hypoxia (Fig. 2, lanes 4—6), indicating anti-inflammatory activity of emodin in synoviocytes.

Emodin Downregulated Hypoxia-Induced Angiogenic Molecules in IL-1β and LPS-Stimulated RA Synoviocytes Hypoxia mediates the secretion of VEGF, an angiogenic factor, leading to the release of proinflammatory mediators in the RA synovium.[3] Thus, the expressions of proinflammatory mediators were analyzed at protein (secreted) and mRNA levels. As shown in Fig. 3, IL-1β and LPS significantly increased the secreted levels of prostagladin E_2 (PGE_2) (product of COX-2) (A), VEGF (B), MMP-1 (C), and MMP-13 (D) under hypoxia by ELISA (lane 3). In contrast, emodin significantly decreased IL-1β and LPS-induced production of the proinflammatory mediators in a dose-dependent manner (Fig. 3, lanes 4—6). Consistent with the results of ELISA, RT-PCR revealed that emodin inhibited IL-1β and LPS-stimulated expression of COX-2, VEGF, HIF-1α, MMP-1, and MMP-13 at the mRNA level under hypoxia (Fig. 4).

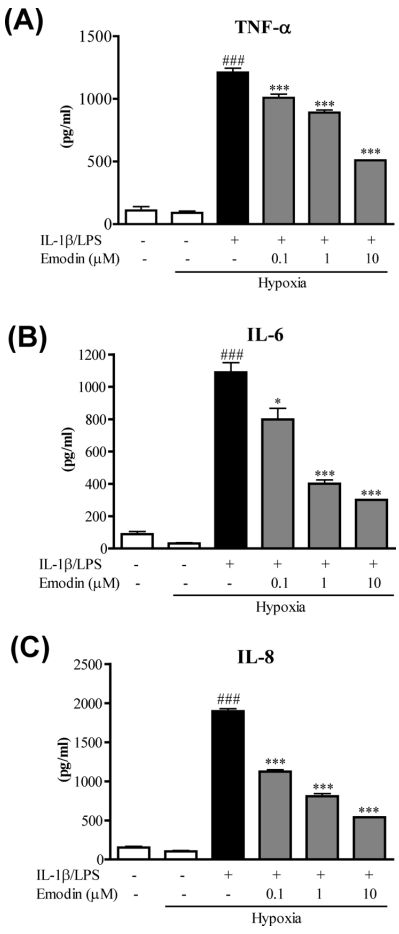

Fig. 2. Effects of Emodin on the Levels of Proinflammatory Cytokines in IL-1β and LPS-Stimulated Synoviocytes

Cells were stimulated with IL-1β and LPS, and treated with various concentrations of emodin (0, 0.1, 1 or 10 μM) for 24 h under hypoxia. The levels of cytokines TNF-α (A), IL-6 (B) and IL-8 (C) were determined by ELISA. Data shown are representative from three independent experiments. Values are expressed as means±standard error (S.E.). ### $p<0.001$ *vs.* untreated control under hypoxia. * $p<0.05$, *** $p<0.001$ *vs.* IL-1β and LPS-stimulated control under hypoxia.

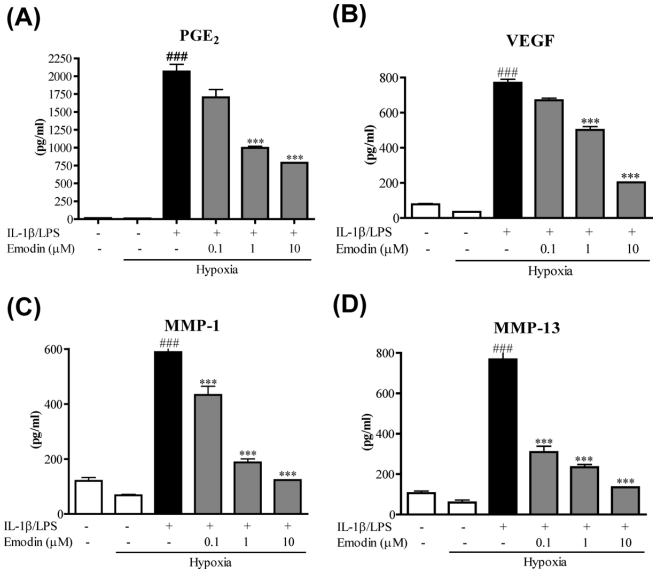

Fig. 3. Effects of Emodin on the Secreted Protein Levels of MMP-1 and -13 in IL-1β and LPS-Stimulated Synoviocytes

Cells were stimulated with IL-1β and LPS, and treated with various concentrations of emodin (0, 0.1, 1 or 10 μM) for 24 h under hypoxia. The levels of cytokines PGE_2 (A), VEGF (B), MMP-1 (C) and MMP-13 (D) were determined by ELISA. Data shown are representative from three independent experiments. Values are expressed as means±standard error (S.E.). ### $p<0.001$ *vs.* untreated control under hypoxia. *** $p<0.001$ *vs.* IL-1β and LPS-stimulated control under hypoxia.

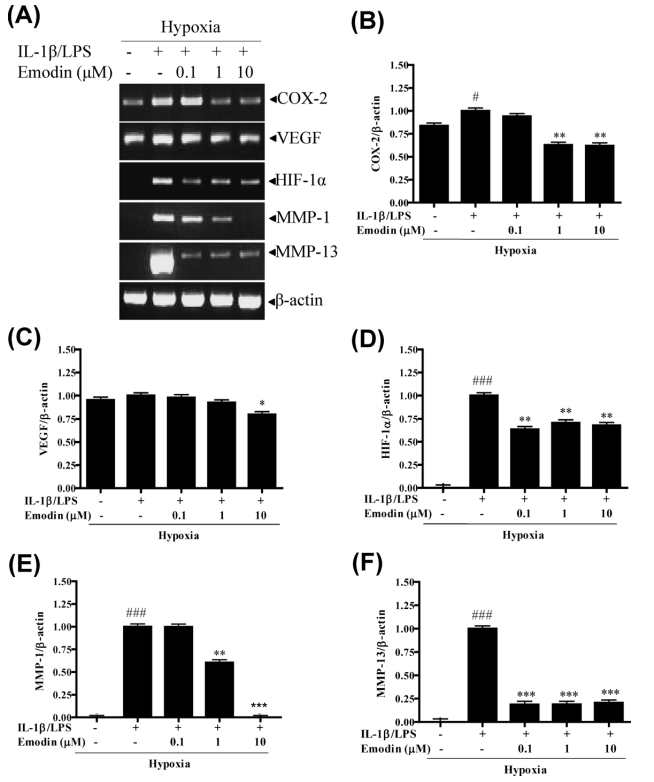

Fig. 4. Effects of Emodin on the mRNA Expression of MMP-1 and -13 in IL-1β and LPS-Stimulated Synoviocytes

Cells were stimulated with IL-1β and LPS, and treated with various concentrations of emodin (0, 0.1, 1 or 10 μM) for 24 h under hypoxia. (A) The gene expressions of COX-2, VEGF, HIF-1α, MMP-1 and MMP-13 were determined by RT-PCR. (B—F) Bar graphs represent the band intensities of PCR products COX-2 (B), VEGF (C), HIF-1α (D), MMP-1 (E) and MMP-13 (F) adjusted by β-actin. Data shown are representative from three independent experiments. Values are expressed as means±standard error (S.E.). $^{\#}p<0.05$ and $^{\#\#\#}p<0.001$ *vs.* untreated control under hypoxia. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ *vs.* IL-1β and LPS-stimulated control under hypoxia.

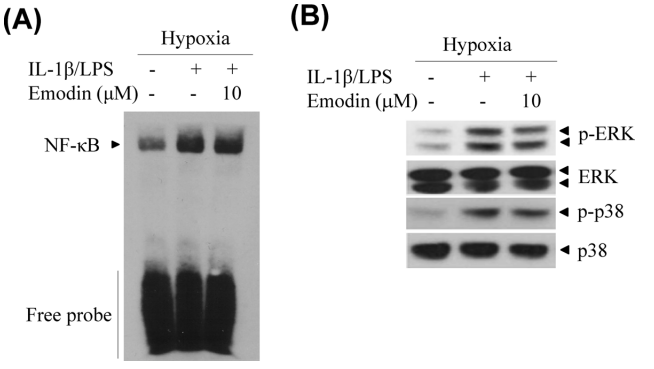

Fig. 5. Effects of Emodin on NF-κB and MAPK in IL-1β and LPS-Stimulated Synoviocytes

Cells were treated with or without emodin (10 μM) in the presence of IL-1β and LPS for 24 h under hypoxia. (A) Nuclear extracts were prepared and subjected to EMSA to determine the activity of NF-κB/DNA binding. (B) Whole cell lysates were prepared and subjected to Western blotting for p-ERK, ERK, p-p38 and p38.

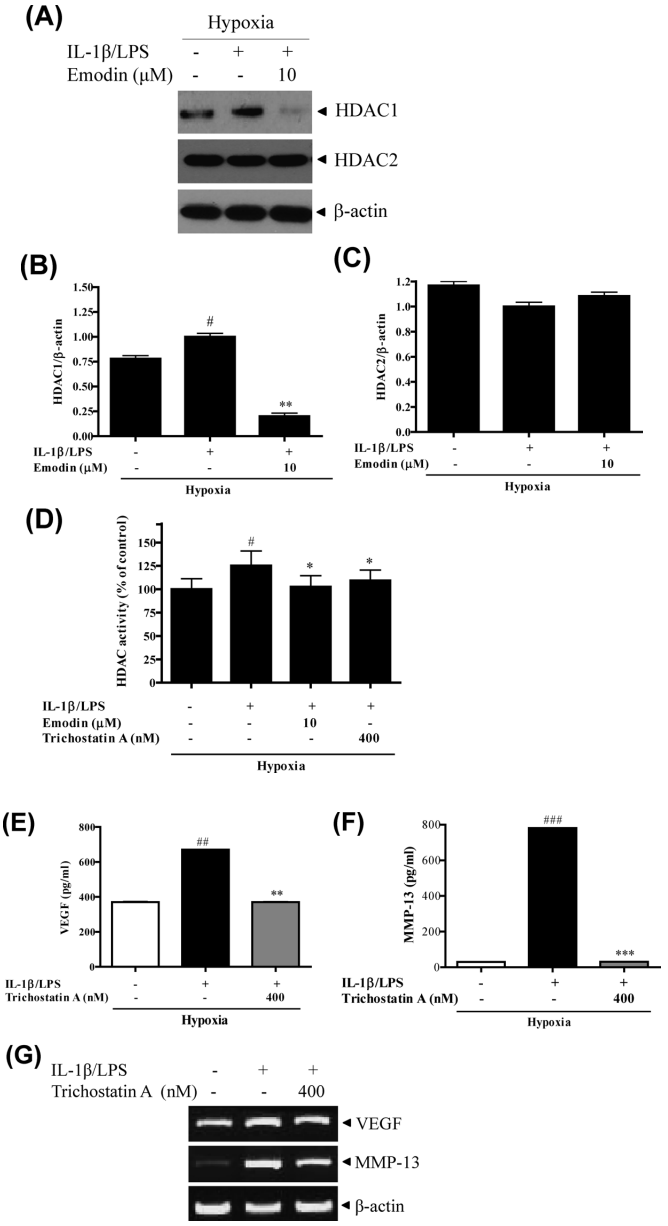

Fig. 6. Effects of Emodin on HDAC 1 in IL-1β and LPS-Stimulated Synoviocytes

Cells were stimulated with IL-1β and LPS, and treated with or without emodin (10 μM). Nuclear extracts were prepared by using NE-PER nuclear extraction kit (Thermo Scientific). (A) Western blotting was performed for HDAC1 and 2. Bar graphs represent the band intensities of protein expression for HDAC1 (B) and HDAC2 (C) adjusted by β-actin. (D) Cells were treated with emodin (10 μM) or trichostatin (400 nM) in the presence of IL-1β and LPS for 24 h under hypoxia. HDAC activity was measured by using HDAC assay kit (Millipore). (E—G) Cells treated with IL-1β and LPS were exposed to trichostatin (400 nM) for 24 h under hypoxia. Culture supernatants were collected and applied ELISA for VEGF (E) and MMP-13 (F). RT-PCR was performed using isolated total RNA for VEGF and MMP-13 (G). Data shown are representative from three independent experiments. Values are expressed as means±standard error (S.E.). $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ *vs.* untreated control under hypoxia. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ *vs.* IL-1β and LPS-stimulated control under hypoxia.

Emodin Suppressed Inflammatory Responses *via* Inhibition of HDAC1, but Not HDAC2, in Hypoxic IL-1β and LPS-Stimulated Synoviocytes Several recent studies reported that expression of inflammation mediators MMP-1 or -13 is associated with activation of NF-κB and mitogen-activated protein kinases (MAPKs) in IL-1β-stimulated RA synoviocytes.[24,25] However, our study showed that emodin did not affect the NF-κB/DNA binding activity (Fig. 5A) as well as phosphorylation of ERK and p38 MAPK (Fig. 5B) in IL-1β and LPS-treated cells.

HDAC is also an important transcription factor to involve in the regulation of inflammatory response in RA.[13,26,27] We found that IL-1β and LPS stimulation increased HDAC1 expression, while emodin remarkably reduced IL-1β and LPS-mediated expression of HDAC1, but not HDAC2, in the

synoviocytes (Figs. 6A—C). In addition, emodin significantly attenuated HDAC transcriptional activity induced by IL-1β and LPS (Fig. 6D), indicating that anti-inflammatory activity of emodin is associated with the transactivation of HDAC1, but not HDAC2 in synoviocytes.

To further confirm the involvement of HDAC in the inhibitory effect of emodin on inflammation-related factors, synoviocytes were treated with emodin or trichostatin A as a known HDAC inhibitor in the presence of IL-1β and LPS. The effects of trichostatin A on VEGF and MMP-13 were analyzed by ELISA and RT-PCR. Trichostatin A significantly suppressed VEGF and MMP-13 levels at secreted protein (Figs. 6E, F) and mRNA levels (Fig. 6G).

DISCUSSION

Although many anti-rheumatic drugs (DMARDs) such as methotrexate and celecoxib, and tumor necrosis factor (TNF) inhibitors have been developed to reduce synovitis (an inflammation of the synovial membrane) and systemic inflammation, the side effects such as vomiting, nausea, diarrhea and liver damage induced by these agents limited their efficacies. Thus, novel therapeutic agents with high efficiency and little side effect are still required for RA therapy.

In the present study, we investigated whether emodin, an active component from the root and rhizomes of *Rheum palmatum* L., exerts anti-inflammatory response in synoviocytes. The cells were exposed to hypoxia before treatment of IL-1β and LPS and/or emodin since hypoxia is a prominent feature in RA synovium.[28] Emodin inhibited the proliferation of synoviocytes in the absence or presence of LPS and IL-1β against synoviocytes, while it did not exert cytotoxic effect in Raw264.7 macrophages and human umbilical vein endothelial cells (HUVECs) (data not shown). Since synoviocytes from RA patient is pathological inflammatory cells regardless of LPS and IL-1β treatment, the inhibitory effects of emodin on inflammatory responses may not be only by the cytotoxic activity of emodin.

In addition, emodin significantly reduced IL-1β and LPS-induced production of proinflammatory cytokines such as TNF-α, interleukin (IL)-6 and -8 in a dose-dependent manner. Also, emodin decreased mRNA expression of cyclooxygenase 2 (COX-2) and level of prostaglandin E_2 (PGE_2), a major product of COX-2 in a dose-dependent manner in the synoviocytes, suggesting inhibitory effects of emodin on proinflammatory cytokines and molecules.

Synovial angiogenesis is a hallmark of RA and can be mediated by VEGF, HIF-1α as well as proinflammatory cytokines.[29,30] Targeting VEGF using IMC-1C11, a chimeric VEGF receptor (VEGFR)-2 and anti-VEGF antibody inhibited synovial endothelial cell proliferation and suppressed arthritis in collagen-induced arthritis mouse model, respectively.[31,32] Recently, several studies reported that several natural products can modulate the inflammation system *via* inhibition of VEGF as well as proinflammatory cytokines in synoviocytes. Lee and colleagues reported that fisetin significantly decreased VEGF as well as TNF-α, IL-6 and -8 in synoviocytes.[19] Also, Cinelli and colleagues reported that RA synoviocytes treated with piascledine revealed lower levels of VEGF compared to untreated control.[33] Likewise, Zheng and colleagues reported that paeoniflorin suppressed the production of VEGF, IL-6, PGE_2 and IL-1 in rats with adjuvant arthritis.[34] In the current study, emodin also significantly inhibited secretion of VEGF in IL-1β and LPS-treated synoviocytes under hypoxia in a dose-dependent manner. Consistently, emodin dose-dependently reduced the mRNA expression of VEGF and HIF-1α in IL-1β and LPS-stimulated synoviocytes, implying emodin exhibits anti-inflammatory activity *via* inhibition of angiogenic biomarkers such as VEGF and HIF-1α.

Matrix metalloproteinases (MMPs) work on proinflammatory cytokines, chemokines and other factors to regulate inflammation and immunity.[35] There are many evidences that synoviocytes produce MMPs, especially MMP-1 and -13 (collagenases), and contribute to joint destruction in RA.[36—38] In our study, IL-1β and LPS treatment significantly induced MMP-1 and -13 expressions at secreted protein and mRNA levels, consistent with the previous papers.[39,40] Transcriptional activation of MMP-1 and -13 can be regulated by various signaling pathways.[41] Mitogen-activated kinases (MAPKs) including ERK, JNK and p38 MAPK induce transcriptional activation of MMP genes.[42,43] Another important transcriptional regulator of MMPs is NF-κB. Pyrrolidine dithiocarbamate (PDTC), an NF-κB inhibitor, up-regulated MMP-1 and MMP-13 in IL-1β-stimulated synoviocytes, suggesting the importance of NF-κB in PDTC-induced anti-inflammatory regulation *via* inhibition of MMP-1 and -13.[44] Thymoquinone, a major constituent of Nigella sativa oil, inhibited IL-1β-induced MMP-1, -3 and -13, and NF-κB p65 level as well as its nuclear translocation in osteoarthritis.[45] In our study, we did not find any significant effect of emodin on IL-1β and LPS-mediated activation of MAPK and NF-κB, indicating that anti-inflammatory activity of emodin is independent of MAPK and NF-κB pathways.

Recent studies suggest that histone deacetylases (HDACs) are implicated in MMP regulation in inflammatory system.[26,46,47] Trichostatin A, a HDAC inhibitor, suppressed synovial inflammation and subsequent cartilage destruction in a collagen antibody-induced arthritis mouse model.[26] In this regard, we examined whether or not emodin can influence HDAC expression and activity in IL-1β and LPS-treated synoviocytes under hypoxia. Our data showed that emodin remarkably suppressed HDAC activity and attenuated the expression of HDAC1, but not HDAC2 in synoviocytes, suggesting the important role of HDAC1 in anti-inflammatory signaling activation in RA synoviocytes. Consistently, trichostatin A treatment confirmed the importance of HDAC inhibition in emodin-mediated anti-inflammatory responses in synoviocytes. Similarly, trichostatin A significantly reduced IL-1β and LPS-induced VEGF and MMP-1 levels as well as HDAC activity in synoviocytes. However, further studies are required to confirm the precise role of HDAC1 in emodin-induced anti-inflammatory activity *in vivo*.

In summary, emodin has potential to inhibit pro-inflammatory cytokines and mediators, and angiogenic factor VEGF. Emodin also suppresses MMP-1, MMP-13 and HDAC1 in RA synoviocytes. Overall, our findings suggest that emodin inhibits proinflammatory cytokines and VEGF productions, and HDAC1 activity in hypoxic RA synoviocytes.

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