Cilostazol inhibits the expression of hnRNP A2/B1 and cytokines in human dermal microvascular endothelial cells

Y. An[1], Z. Zheng[2], X. Zhang[3], S.B. Cho[4], D.Y. Kim[5], M.J. Choi[5], D. Bang[4]

[1]Department of Dermatology, Yanbian University Hospital, Yanji, China;
[2]Department of Cardiology, Affiliated Zhongshan Hospital of Dalian University, Dalian, China;
[3]Department of Pathology, Yanbian University Hospital, Yanji, China;
[4]Department of Dermatology and Cutaneous Biology Research Center, International St. Mary's Hospital, Catholic Kwandong University, College of Medicine, Incheon, Korea;
[5]Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul, Korea.

Yinghua An, MD, PhD*
Zhenguo Zheng, MD, PhD*
Xianglan Zhang, MD, PhD
Sung Bin Cho, MD, PhD
Do Young Kim, MD
Min Ju Choi, MD
Dongsik Bang, MD, PhD

*These authors contributed equally to this work.

Please address correspondence and reprint requests to:
Dr Dongsik Bang,
Department of Dermatology, International St. Mary's Hospital, Catholic Kwandong University, College of Medicine,
25 Simgok-ro, Seo-gu,
22711 Incheon, Republic of Korea.
E-mail: dbang@yuhs.ac

Received on December 15, 2016; accepted in revised form on June 8, 2017.

Clin Exp Rheumatol 2017; 35 (Suppl. 108): S60-S66.

Key words: hnRNP A2/B1, cilostazol, Behçet's disease, endothelial cell

Funding: this study was supported by research funds from Korea Otsuka Pharmaceutical Co., Ltd., and by research funding from Catholic Kwandong University, International St. Mary's Hospital (grant no. CKURF-20140683000).

Competing interests: none declared.

ABSTRACT

Objective. *hnRNP A2/B1 has been identified as a target antigen of anti-endothelial cell IgA antibody in patients with Behçet's disease (BD). In addition, increased expression of cellular hnRNP A2/B1 is stimulated by Streptococcus sanguinis or the sera from patients with BD. We aimed to investigate the effects of cilostazol on the expression of hnRNP A2/B1 and chemokines in human dermal microvascular endothelial cells (HDMECs).*

Methods. *Expression of hnRNP A2/B1, cytokines, and chemokines in HDMECs was induced by tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and lipopolysaccharide (LPS). HDMECs were treated with cilostazol (10 μM) and the inhibitory effects were evaluated with real-time polymerase chain reaction and immunocytochemistry.*

Results. *Expression of hnRNP A2/B1, CXCL1, CXCL2, CXCL8, and IL-1β mRNA was significantly increased in HDMECs treated with all three stimulants. In addition, mRNA expression of hnRNP A2/B1 and inflammatory mediators was significantly inhibited in HDMECs treated with various stimulants with cilostazol pretreatment. Immunocytochemistry demonstrated that cilostazol pretreatment effectively inhibited the stimulant-induced increased expression of hnRNP A2/B1 in the nucleus and cytoplasm of HDMECs.*

Conclusions. *Cilostazol pretreatment can reduce the excessive expression of inflammatory cytokines and chemokines and hnRNP A2/B1 by the BD-related stimulants, including TNF-α, IL-1β, and LPS, in HDMECs. We suggest that cilostazol may have therapeutic efficacy in inhibiting the major inflammatory reaction in the pathogenesis of BD.*

Introduction

Behçet's disease (BD) is a chronic multifactorial vasculitis that mainly affects small blood vessels in various organs (1). Its susceptibility was proved to be strongly associated with the HLA-B*51 antigen (2, 3). Although the exact pathogenesis of BD remains enigmatic, endothelial cells are suggested as a primary target in this disease, and α-enolase and hnRNP A2/B1 have been identified as target antigens of anti-endothelial cell IgM and IgA antibodies, respectively (4, 5). Our study group recently demonstrated that cellular hnRNP A2/B1 and hnRNP A2/B1 mRNA were increased by the stimuli of *Streptococcus sanguinis* or the sera from patients with BD. In addition, we observed that these stimuli induced membrane expression of hnRNP A2/B1, which is usually detected in the cytoplasm and nucleus under healthy conditions, in human dermal microvascular endothelial cells (HDMECs) (6).

Cilostazol is a specific inhibitor of 3'-5'-cyclic adenosine monophosphate (cAMP) phosphodiesterase type III (PDE3) (7). Cilostazol is known to inhibit platelet aggregation and act as a vasodilator, useful in the treatment of peripheral vascular disease (7, 8). In addition, cilostazol can decrease the expression of monocyte chemoattractant protein-1 (MCP-1) and C-C chemokine receptor-2 (CCR2) as well as vascular cell adhesion molecule-1 (VCAM-1) in vascular endothelial cells to inhibit leukocyte-endothelial cell interaction (9). In diabetic rats, cilostazol protects vascular inflammation through prevents the overexpression of VCAM-1 via inhibiting the activation of nuclear factor-κB (10, 11).

In the present study, we investigated the effects of cilostazol on the expression of hnRNP A2/B1 and chemokines in HDMECs. Expression of hnRNP A2/B1 and chemokines in HDMECs was induced by tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and lipopolysaccharide (LPS). HDMECs were pretreated with cilostazol (10

μM), and the inhibitory effects were evaluated with real-time polymerase chain reaction (PCR) and immunocytochemistry.

Materials and methods

Cell culture

Purchased HDMECs (Lonza Group Ltd., Basel, Switzerland) were prepared and treated in a tissue incubator using endothelial basal medium-2 (Lonza Group Ltd.) containing human epidermal growth factor, hydrocortisone, vascular endothelial growth factor, human fibroblast growth factor-β, gentamicin, amphotericin B, R3-insulin like growth factor-1, ascorbic acid, and 2% fetal bovine serum. HDMECs were subcultured serially from passage 3 to 10 at 37°C in a CO_2 incubator.

Chemicals and cell stimulation

Cilostazol (OPC-13013) (6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2-(1H)-quinolinone) was generously donated by Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan) and dissolved in dimethyl sulfoxide as a 10 mM stock solution. LPS (*E. coli* O55:B5; Santa Cruz Biotechnology, Santa Cruz, CA, USA), TNF-α (R&D Systems, Minneapolis, MN, USA), and IL-1β (R&D Systems) were purchased. As described previously (12), cilostazol 10 μM was added to confluent HDMECs with endothelial basal medium-2 with 2% fetal bovine serum, growth factors, and antibiotics for the indicated times before the addition of LPS 200 ng/mL, TNF-α 100 ng/mL, and IL-1β 10 ng/mL. All experiments were performed in triplicate.

Cell viability assay

Cell viability was assessed by the methylthiazoletetrazolium (MTT) conversion test. Briefly, HDMECs were seeded with 20,000 cells/well in 96-well gelatin-coated tissue culture plates. Confluent HDMECs received endothelial basal medium-2 with 2% fetal bovine serum, growth factors, and antibiotics, 5 h prior to stimulation with or without cilostazol for 12, 24, and 48 h. After incubation, 20 μL/well of MTT solution (Sigma-Aldrich, St Louis, MO, USA) at a concentration of 5 mg/mL diluted with phosphate-buffered saline (PBS) was added, and the cells were incubated for 2h. The medium was aspirated and replaced with 150 μL/well of ethanol/dimethyl sulfoxide solution (1:1). The plates were shaken for 20 min, and the optical density measured at 570 to 630 nm using enzyme-linked immunosorbent assay (ELISA) (Bio-Tek Instruments, Inc., Winooski, VT, USA).

Table I. Polymerase chain reaction primers used in this experiment.

Name		Sequence (5' to 3')
ACTB	F	ATAGCACAGCCTGGATAGCAACGTAC
	R	CACCTTCTACAATGAGCTGCGTGTG
CXCL-1	F	ATTCACCCCAAGAACATCCA
	R	GATGCAGGATTGAGGCAAG
CXCL-2	F	GCAGGGAATTCACCTCAAGA
	R	GACAAGCTTTCTGCCCATTC
CXCL-8	F	CGGAAGGAACCATCTCACTG
	R	ACTCCTTGGCAAAACTGCAC
IL-1β	F	GCTGAGGAAGATGCTGGTTC
	R	TCGTTATCCCATGTGTCGAA

Immunocytochemistry

For the assessment of hnRNP A2/B1 expression, HDMECs were stimulated with LPS, IL-1β, and TNF-α for 6, 12, or 24 h. In a Lab-Tek chamber, 2 X 10^4 cells from each group were cultured and fixed with 4% paraformaldehyde, followed by 1h incubation at room temperature with 1:200 diluted mouse anti-human hnRNP A2/B1 antibody (Abcam, Cambridge, UK). Cells were washed 3 times with PBS solution, after which a secondary antibody (Real™ Envision™ HRP Rabbit/Mouse, DAKO, Carpinteria, CA, USA) was added with a 30-min incubation at room temperature. Cells were counterstained with haematoxylin for 1 min, and after mounting, the expression of hnRNP A2/B1 was examined in each group.

Real-time PCR

Total RNA was isolated with TriZol reagent (Canadian Life Technologies, Burlington, Ontario, Canada) and the cDNA was synthesised using SuperScript II RNase H Reverse Transcriptase (Canadian Life Technologies). The primers (Bioneer, Seoul, Korea) used are indicated in Table I. Gene expression was monitored using ABI 7500 software (Applied Biosystems, Foster City, CA, USA) according to the standard procedure. The real-time PCR cycles included 40 cycles of general denaturation at 94°C for 30 sec, annealing, and elongation at 60°C for 1 min, except for the first cycle with a 15-min denaturation and the last cycle with a 7-min elongation at 72°C. Real-time polymerase quantification of the signals was performed by normalising the gene signals with β-actin signal. All experiments were performed in triplicate.

Statistical analysis

The data were presented as mean ± standard deviation (SD), and Wilcoxon signed rank test was used to analyse the differences in the levels of hnRNP A2/B1, CXCL1, CXCL2, CXCL8, and IL-1β depending on each subcellular fraction of each group. All analyses were performed using the Statistical Package for the Social Sciences v. 18.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant when the *p*-value was less than 0.05.

Results

Effect of cilostazol on cell viability of HDMECs

To determine the cytotoxicity of cilostazol in HDMEC cells, the viability of the cells treated with the indicated concentrations of cilostazol was detected by MTT assay. Cilostazol caused minimal toxicity to HDMECs at the indicated concentrations, and 10 μM of cilostazol showing 87.8% viability for HDMECs was used in this study (Fig. 1).

Inhibitory effect of cilostazol on inflammatory factor expression induced by various stimulants in HDMECs

First, HDMECs were treated with various stimulants including TNF-α, IL-

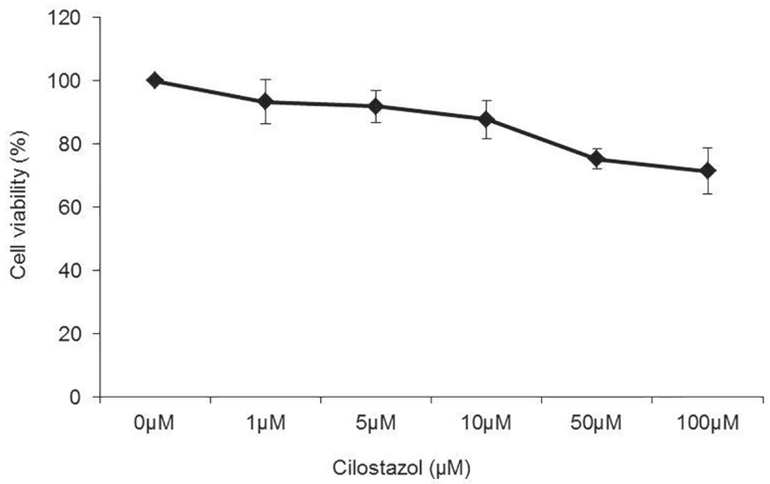

Fig. 1. Cells from the human endothelial cell line, human dermal microvascular endothelial cells (HDMECs), were serum-starved and treated with different concentrations of cilostazol (0, 1, 5, 10, 50, 100 μM) for 72h. The indicated concentrations of cilostazol showed minimal toxicity for HDMECs. A relatively lower dose (10 μM) of cilostazol induced 87.8% viability for HDMECs and was used in this study. Bars, standard deviation (SD).

1β, and LPS, then the level of mRNA expression was comparatively investigated in cells with or without treatment. All of the stimulants significantly induced mRNA expression of all three chemokines and IL-1β in HDMECs at each indicated time point. Next, mRNA expression of all inflammatory factors was comparatively investigated in stimulated HDMECs with and without treatment with cilostazol.

A significant inhibitory effect on the expression of inflammatory factors was observed in different groups of HDMECs regardless of the stimulants. In the TNF-α-treated group, both CXCL1 and IL-1β expression were significantly reduced by cilostazol treatment at each indicated time point. Moreover, reduced CXCL2 and CXCL8 expression was observed from 12 h and 24 h after cilostazol treatment, respectively (Fig. 2). In the LPS-treated group, cilostazol treatment induced significantly reduced CXCL8 expression at each of the indicated time points. Moreover, expression of CXCL1, CXCL2, and IL-1β was also significantly decreased at the 12-h and 24-h time points by cilostazol treatment (Fig. 3). In the IL-1β treated group, cilostazol treatment reduced CXCL1 expression at the 12-h and 24-h time points. CXCL8 expression was also decreased at the 6-h and 12-h time points by cilostazol treatment. Moreover, decreased expression of both CXCL2 and IL-1β was observed at the 6-h, 12-h, and 24-h time points under cilostazol treatment (Fig. 4).

Inhibitory effect of cilostazol on hnRNP A2/B1 expression in HDMECs

To further investigate the influence of cilostazol on expression of hnRNP A2/B1, we examined hnRNP A2/B1 mRNA and protein expression in stimulated HDMECs with or without cilostazol treatment. Significantly increased expression of hnRNP A2/B1 mRNA was induced by all three stimulants. An inhibitory effect of cilostazol on hnRNP A2/B1 mRNA expression was observed at the 6-h, 12-h, and 24-h time points in the TNF-α-, LPS-, and IL-1β-treated groups (Fig. 5).

Regarding hnRNP A2/B1 protein, HDMECs showed increased expression

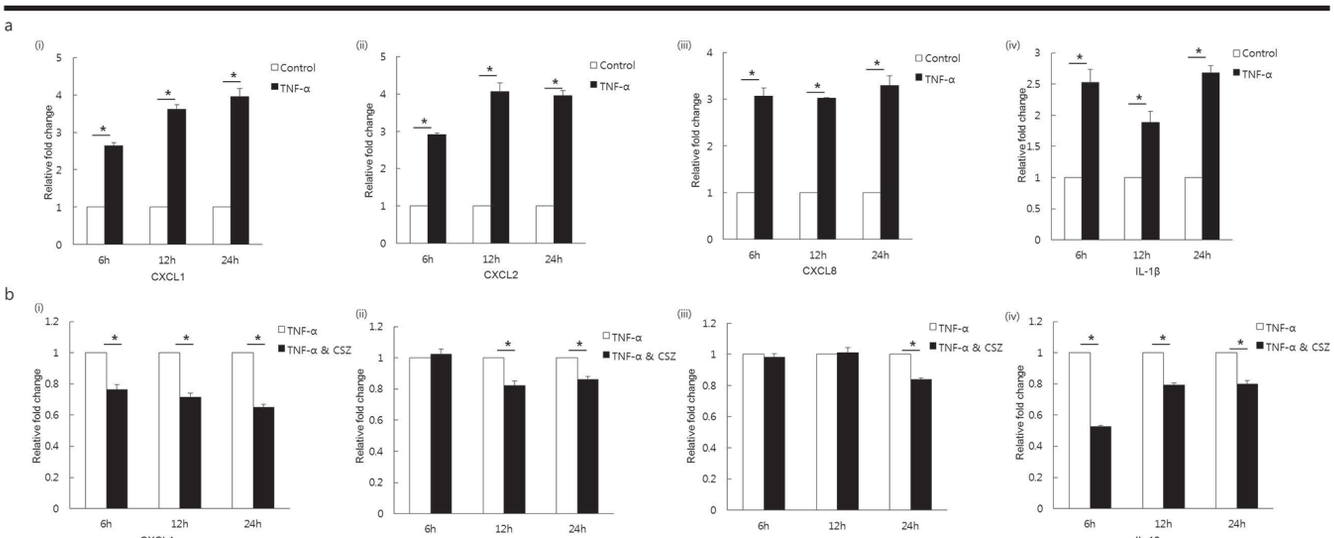

Fig. 2. HDMECs treated with TNF-α by different time points. (**a**) Compared to control group, significantly increased mRNA expression of all three chemokines and IL-1β were found in TNF-α treated group at each indicated time point; The inhibitory effect of cilostazol on expression of inflammatory factors in HDMECs treated with TNF-α. (**b**) Both CXCL1 (i) and IL-1β (iv) expression was significantly reduced by cilostazol treatment at each indicated time point. Moreover, reduced CXCL2 (ii) and CXCL8 (iii) expression was found from 12h and 24h after cilostazol treatment, respectively. Control group: HDMECs incubated with supplemented endothelial cell medium. Quantitative results indicate average values of three independent experiments, each of which was performed in triplicate. The results are shown as mean values±SD (n=9) and were analysed by the Wilcoxon signed rank test. *$p \leq 0.05$.

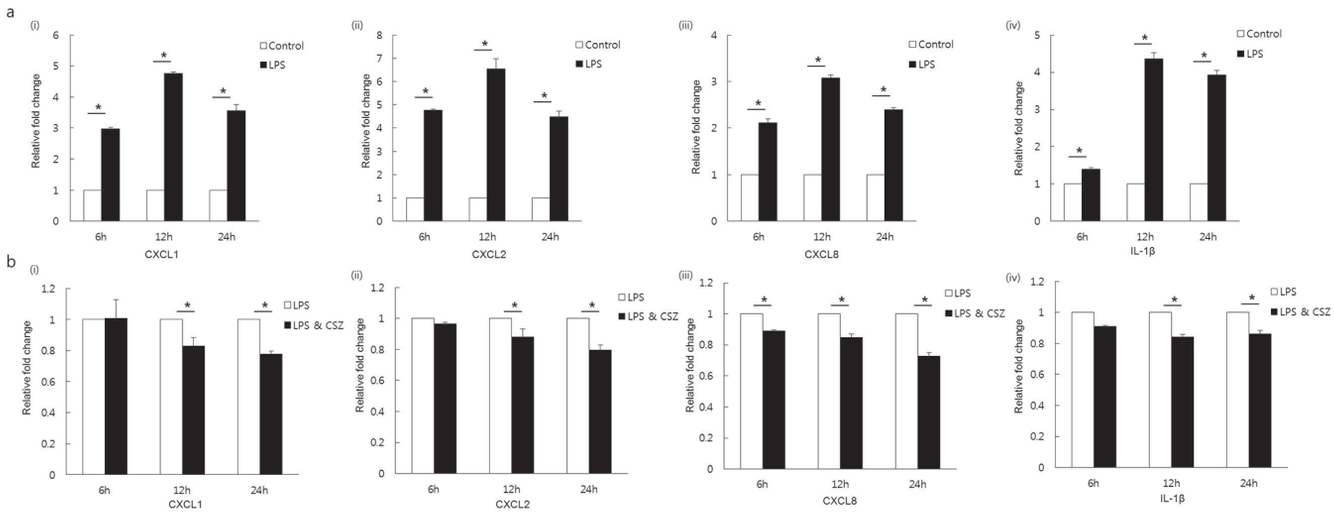

Fig. 3. HDMECs treated with LPS by different time points. (**a**) Compared to control group, significantly increased mRNA expression of all three chemokines and IL-1 β were found in LPS treated group at each indicated time point; Inhibitory effect of cilostazol on expression of inflammatory factors in HDMECs treated with LPS. (**b**) CXCL1 (i), CXCL2 (ii) and IL-1β (iv) expression was significantly decreased at the 12-h and 24-h time points by cilostazol treatment. CXCL8 (iii) expression was significantly reduced at each indicated time point. Control group: HDMECs incubated with supplemented endothelial cell medium. Quantitative results indicate average values of three independent experiments, each of which was performed in triplicate. The results are shown as mean values±SD and were analysed by the Wilcoxon signed rank test. *$p \leq 0.05$.

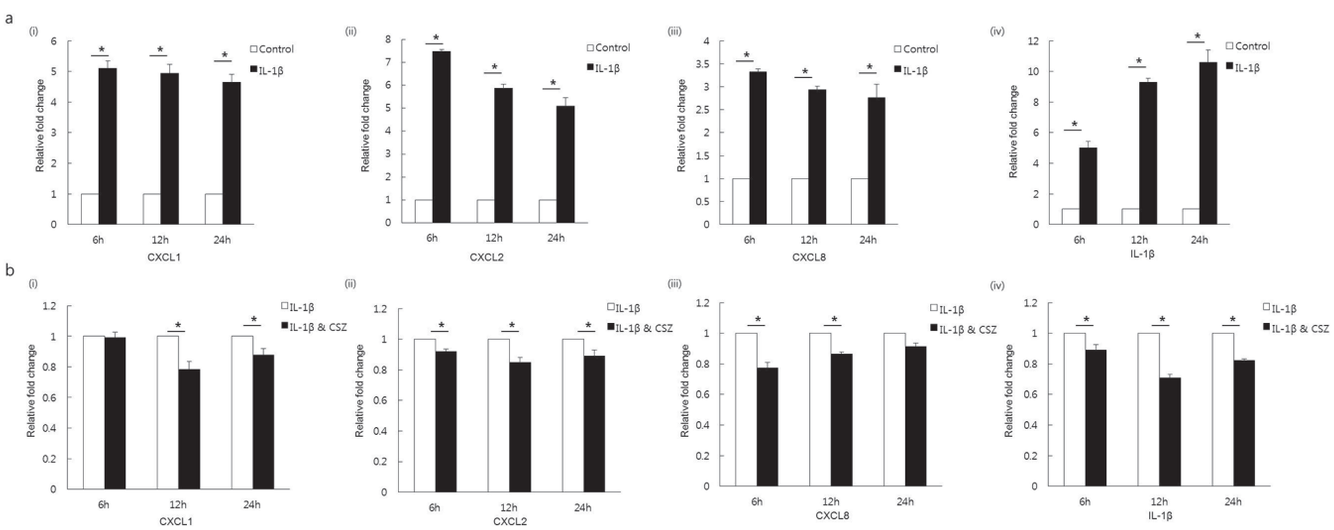

Fig. 4. HDMECs treated with IL-1β by different time points. (**a**) Compared to control group, significantly increased mRNA expression of all three chemokines and IL-1β were found in IL-1β treated group at each indicated time point; Inhibitory effect of cilostazol on expression of inflammatory factors in HDMECs treated with IL-1β. (**b**) After cilostazol treatment, (i) CXCL1 expression was reduced at the 12-h and 24-h time points and (iii) CXCL8 expression was also decreased at the 6-h and 12-h time points. Decreased expression of both (ii) CXCL2 and (iv) IL-1β was found at the 6-h, 12-h, and 24-h time points under cilostazol treatment. Control group: HDMECs incubated with supplemented endothelial cell medium. Quantitative results indicate average values of three independent experiments, each of which was performed in triplicate. The results are shown as mean values±SD and were analysed by the Wilcoxon signed rank test. *$p \leq 0.05$

due to treatment with any stimulants. Normally, hnRNP A2/B1 protein is weakly expressed in the cytoplasm and nucleus of HDMECs. However, with TNF-α or IL-1β treatment, nuclear expression of hnRNP A2/B1 increased at all indicated time points. Moreover, cytoplasmic expression of hnRNP A2/B1 also significantly increased from 12 h of TNF-α or IL-1β treatment. Cells with LPS treatment also showed increased hnRNP A2/B1 expression at each indicated time point both in the cytoplasm and nucleus. Importantly, increased hnRNP A2/B1 expression that was induced by all three stimulants was sufficiently reduced by cilostazol treatment at all indicated time points (Fig. 6).

Discussion

Many chemokines have been studied extensively in various chronic inflammatory diseases, such as lupus erythematosus, sarcoidosis, and rheumatoid arthritis, and the crucial role of different chemokines in the pathogenesis of the underlying diseases has received a great deal of attention (13). According to an *in vivo* study using gene-deleted mice, the potential synergistic effect of different chemokines largely depended on single or multiple receptors (14). CXC chemokines, including CXCL1,

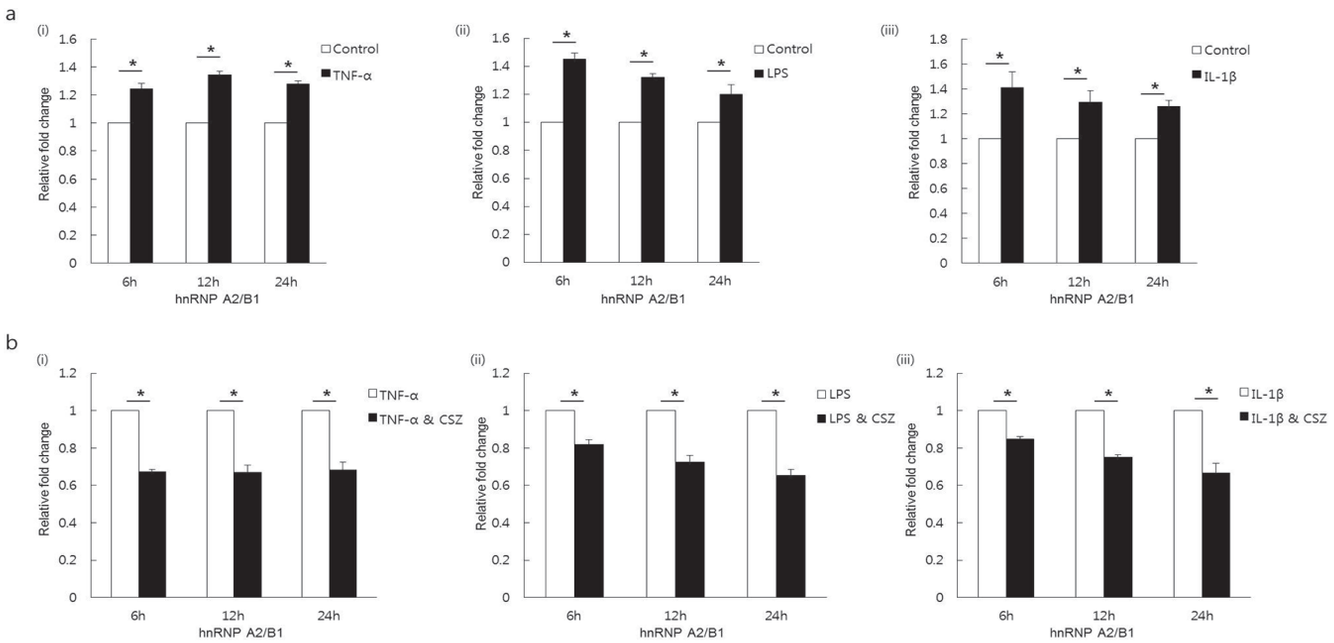

Fig. 5. Significantly increased hnRNP A2/B1 mRNA expression was induced by all three stimulants. (**a**) Inhibitory effect of cilostazol on hnRNP A2/B1 mRNA expression in HDMECs. (**b**) Significantly decreased hnRNP A2/B1 mRNA expression was observed after cilostazol treatment in (i) TNF-α-, (ii) LPS-, and (iii) IL-1β-stimulated HDMECs at each indicated time points. Control group: HDMECs incubated with supplemented endothelial cell medium. Quantitative results indicate average values of three independent experiments, each of which was performed in triplicate. The results are shown as mean values±SD and were analysed by the Wilcoxon signed rank test. *$p \leq 0.05$.

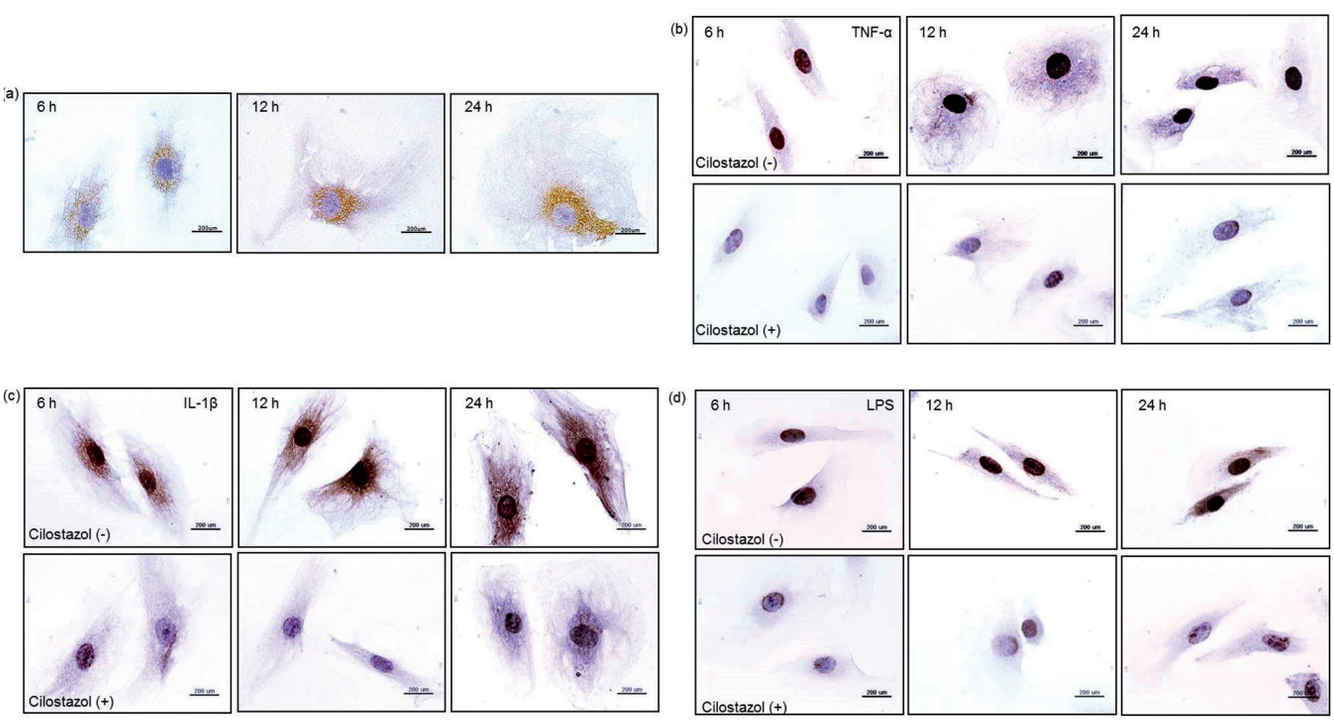

Fig. 6. Immunostaining of hnRNP A2/B1 in HDMECs stimulated with LPS, IL-1β, and TNF-α for 6, 12, and 24 h. (**a**) Compared with HDMECs incubated with supplemented endothelial cell medium, increased expression of cytoplasmic and nuclear cytoplasmic hnRNP-A2/B1 was detected in HDMECs stimulated with these three cytokines. (**b-d**) Increased hnRNP A2/B1 expression that was induced by all three stimulants was sufficiently reduced by cilostazol treatment at all indicated time points.

CXCL2, and CXCL8, also known as neutrophilic chemokines, can chemoattract neutrophils and thereby promote inflammation and the immune response (15, 16). In BD, various cytokines including IL-6, IL-17, IL-18, IL-26, interferon-γ, TNF-α and IL-10 were increased (17-19).

Neutrophil infiltration is one of the major histopathologic features in the inflamed lesions of BD, and increased CXCL1 and CXCL8 expression is also frequently de-

tected in the serum of patients with BD (20, 21). Furthermore, CXCL8 has been known as a biomarker reflecting BD activity (21). These CXC chemokines can be induced by bacterial toxins, such as LPS, along with proinflammatory cytokines, such as TNF-α and IL-1β, in vascular endothelial cells, which have been believed to be a primary target of BD (22). Previous studies reported that expression of TNF-α and IL-1β was increased in the sera of patients with BD, and anti-IL-1β or anti-TNF-α therapy was also effective in BD (23-25).

In the present study, we stimulated HDMECs using TNF-α, IL-1β, and LPS and found that expression of CXC chemokines, including CXCL1, CXCL2, and CXCL8, as well as IL-1β, was markedly increased. Activation or inhibition of chemokine production may be an effective therapeutic approach in different types of diseases related to chemokines, including BD. To abrogate the activation of various chemokines, many chemokine antagonists were developed, and their effectiveness was widely investigated in different types of proinflammatory and autoimmune diseases (26).

HnRNPs are RNA binding proteins, which are present only in the nucleoplasm and cytoplasm under healthy conditions (26). HnRNPs bind with mRNAs and are transported to the cytoplasm from the nucleus, after which some are degraded in the cytoplasm and the others return to the nucleus (26). Cellular hnRNP A2/B1 and hnRNP A2/B1 mRNA were upregulated by the stimuli of the sera from patients with BD or *S. sanguinis* in a previous study (6). We observed that inflammatory stimuli, including TNF-α, IL-1β, and LPS, also induced hnRNP A2/B1 expression in the present study. TNF-α antagonists and anti-IL-1 treatment may be useful elements of the therapeutic plan for the acute and chronic stages of BD as well as the prevention of relapse (27, 28). IL-1β, as mentioned in our previous study, and hnRNP A2/B1 as a target antigen of endothelial cells in patients with BD, may also have important roles in BD pathogenesis (6). Thus, to abrogate production of those CXC chemokines, IL-1β, and hnRNP A2/B1 from sitimulated vascular endothelial cells may actually contribute to BD therapy. At present, although there are numerous therapeutic regimens in BD treatment, such as traditional immunosuppressants, corticosteroids, and various antagonists, some patients with BD still demonstrate resistance to various treatments (29). So, other effective therapeutic approaches still require investigation.

Anti-inflammatory effects of cilostazol have been reported by many investigators (9-12). In the present study, we further investigated whether cilostazol also affects the production of hnRNP A2/B1, IL-1β, and chemokines in stimulated HDMECs. Potent inhibitory effects of cilostazol on the expression of various proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, and CXCL1, in various target cells, were also reported by some investigators (30-32). LPS-induced TNF-α production was significantly reduced by cilostazol treatment in THP-1 cells, RAW 264.7 murine macrophages, and BV2 microglia (33, 34). In BV2 microglia, LPS-induced IL-1β expression was also significantly reduced by cilostazol treatment (34). However, the effect of cilostazol on HDMECs has not been precisely established. In the present study, we found a strong inhibitory effect of cilostazol on CXCL1, CXCL2, CXCL8, and IL-1β mRNA expression in HDMECs treated with various stimulants. Importantly, cilostazol also showed a strong inhibitory effect on both mRNA and protein expression of hnRNP in HDMECs regardless of the stimulant. All of these findings demonstrate that cilostazol effectively inhibits the expression of hnRNP A2/B1 and inflammatory factors in HDMECs when stimulated by TNF-α, IL-1β, and LPS. It can also be postulated that cilostazol may prove useful in treatment modalities for the inflammatory disorder of BD.

In conclusion, our data demonstrated that pretreatment with cilostazol can reduce the excessive expression of inflammatory cytokines and chemokines as well as hnRNP A2/B1 induced by the BD-related stimulants, including TNF-α, IL-1β, and LPS, in HDMECs. We suggest that cilostazol may have therapeutic efficacy in inhibiting the major inflammatory reaction in the pathogenesis of BD. Further experimental validations, including *in vivo* analysis, are required to confirm the effect of cilostazol in BD patients.

Acknowledgements

We thank Prof. Ki-Yoel Kim, PhD (Biostatisticians, Biostatistics Collaboration Unit, Yonsei University College of Medicine, Seoul, Korea) for her help with the statistical analyses.

References

1. SAKANE T, TAKENO M, SUZUKI N, INABA G: Behçet's disease. *N Engl J Med* 1999; 341: 1284-91.
2. YAZICI H: Behçet's syndrome in the 2000s: 'Where is the wisdom we have lost in knowledge?' *Clin Exp Rheumatol* 2016; 34 (Suppl. 102): 23-5.
3. SAZZINI M, GARAGNANI P, SARNO S *et al*.: Tracing Behçet's disease origins along the Silk Road: an anthropological evolutionary genetics perspective. *Clin Exp Rheumatol* 2015; 33 (Suppl. 94): S60-6.
4. LEE KH, CHUNG HS, KIM HS *et al*.: Human alpha-enolase from endothelial cells as a target antigen of anti-endothelial cell antibody in Behçet's disease. *Arthritis Rheum* 2003; 48: 2025-35.
5. CHO SB, AHN KJ, KIM DH *et al*.: Identification of HnRNP-A2/B1 as a target antigen of anti-endothelial cell IgA antibody in Behçet's disease. *J Invest Dermatol* 2012; 132: 601-8.
6. CHO SB, ZHENG Z, AHN KJ *et al*.: Serum IgA reactivity against GroEL of Streptococcus sanguinis and human hnRNP A2/B1 in patients with Behçet's disease. *Br J Dermatol* 2013; 168: 977-83.
7. WEINTRAUB WS: The vascular effects of cilostazol. *Can J Cardiol* 2006; 22: 56B-60B.
8. SCHROR K: The pharmacology of cilostazol. *Diabetes Obes Metab* 2002; 4: S14-9.
9. PARK SY, LEE JH, KIM CD *et al*.: Cilostazol suppresses superoxide production and expression of adhesion molecules in human endothelial cells via mediation of cAMP-dependent protein kinase-mediated maxi-K channel activation. *J Pharmacol Exp Ther* 2006; 317: 1238-45.
10. MORI D, ISHII H, KOJIMA C, NITTA N, NAKAJIMA K, YOSHIDA M: Cilostazol inhibits monocytic cell adhesion to vascular endothelium via upregulation of cAMP. *J Atheroscler Thromb* 2007; 14: 213-8.
11. GAO L, WANG F, WANG B *et al*.: Cilostazol protects diabetic rats from vascular inflammation via nuclear factor-kappa B-dependent down-regulation of vascular cell adhesion molecule-1 expression. *J Pharmacol Exp Ther* 2006; 318: 53-8.
12. KIM KY, SHIN HK, CHOI JM, HONG KW: Inhibition of lipopolysaccharide-induced apoptosis by cilostazol in human umbilical vein endothelial cells. *J Pharmacol Exp Ther* 2002; 300: 709-15.
13. BAGGIOLINI M: Chemokines in Behçet's

disease, a field to be explored as a potential basis for therapy. *Adv Exp Med Biol* 2003; 528: 239-43.
14. GERARD C, ROLLINS BJ: Chemokines and disease. *Nat Immunol* 2001; 2: 108-15.
15. NAVAS A, VARGAS DA, FREUDZON M, MCMAHON-PRATT D, SARAVIA NG, GOMEZ MA: Chronicity of dermal leishmaniasis caused by Leishmania panamensis is associated with parasite-mediated induction of chemokine gene expression. *Infect Immun* 2014; 82: 2872-80.
16. BOUGARN S, CUNHA P, HARMACHE A, FROMAGEAU A, GILBERT FB, RAINARD P: Muramyl dipeptide synergizes with *Staphylococcus aureus* lipoteichoic acid to recruit neutrophils in the mammary gland and to stimulate mammary epithelial cells. *Clin Vaccine Immunol* 2010; 17: 1797-809.
17. ESATOGLU SN, HATEMI G, LECCESE P, OLIVIERI I: Highlights of the 17th International Conference on Behçet's syndrome. *Clin Exp Rheumatol* 2016; 34 (Suppl. 102): S3-9.
18. HATEMI G, SEYAHI E, FRESKO I, TALARICO R, HAMURYUDAN V: One year in review 2016: Behçet's syndrome. *Clin Exp Rheumatol* 2016; 34 (Suppl. 102): S10-22.
19. WILSON P, LARMINIE C, SMITH R: Literature mining, gene-set enrichment and pathway analysis for target identification in Behçet's disease. *Clin Exp Rheumatol* 2016; 34 (Suppl. 102): S101-10.
20. ERDEM H, PAY S, SERDAR M *et al*.: Different ELR (+) angiogenic CXC chemokine profiles in synovial fluid of patients with Behçet's disease, familial Mediterranean fever, rheumatoid arthritis, and osteoarthritis. *Rheumatol Int* 2005; 26: 162-7.
21. SARUHAN-DIRESKENELI G, YENTUR SP, AKMAN-DEMIR G, ISIK N, SERDAROGLU P: Cytokines and chemokines in neuro-Behçet's disease compared to multiple sclerosis and other neurological diseases. *J Neuroimmunol* 2003; 145: 127-34.
22. WOO MY, CHO O, LEE MJ, KIM K, LEE ES, PARK S: Differential effects of colchicine in blood mononuclear cells of patients with Behçet disease in relation to colchicine responsiveness. *Br J Dermatol* 2012; 167: 914-21.
23. ZHOU ZY, CHEN SL, SHEN N, LU Y: Cytokines and Behçet's disease. *Autoimmun Rev* 2012; 11: 699-704.
24. MESQUIDA M, MOLINS B, LLORENC V *et al*.: Proinflammatory cytokines and C-reactive protein in uveitis associated with Behçet's disease. *Mediators Inflamm* 2014; 2014: 396204.
25. BOUALI E, KAABACHI W, HAMZAOUI A, HAMZAOUI K: Interleukin-37 expression is decreased in Behçet's disease and is associated with inflammation. *Immunol Lett* 2015; 167: 87-94.
26. DREYFUSS G, KIM VN, KATAOKA N: Messenger-RNA-binding proteins and the messages they carry. *Nat Rev Mol Cell Biol* 2002; 3: 195-205.
27. ZLATANOVIC G, JOVANOVIC S, VESELINOVIC D, ZIVKOVIC M: [Efficacy of TNF-alpha antagonist and other immunomodulators in the treatment of patients with ophthalmologic manifestations of Behçet's disease and HLA B51 positive vasculitis]. *Vojnosanit Pregl* 2012; 69: 168-74.
28. BILGINER Y, AYAZ NA, OZEN S: Anti-IL-1 treatment for secondary amyloidosis in an adolescent with FMF and Behçet's disease. *Clin Rheumatol* 2010; 29: 209-10.
29. SAYGIN C, UZUNASLAN D, HATEMI G: Currently used biologic agents in the management of Behçet's syndrome. *Curr Med Chem* 2015; 22: 1976-85.
30. ZHONG TY, ARANCIBIA S, BORN R *et al*.: Hemocyanins Stimulate Innate Immunity by Inducing different temporal patterns of proinflammatory cytokine expression in macrophages. *J Immunol* 2016; 196: 4650-62.
31. KATA D, FOLDESI I, FEHER LZ, HACKLER L, JR., PUSKAS LG, GULYA K: Rosuvastatin enhances anti-inflammatory and inhibits proinflammatory functions in cultured microglial cells. *Neuroscience* 2016; 314: 47-63.
32. HENNESSY E, GRIFFIN EW, CUNNINGHAM C: Astrocytes are primed by chronic neurodegeneration to produce exaggerated chemokine and cell infiltration responses to acute stimulation with the cytokines IL-1beta and TNF-alpha. *J Neurosci* 2015; 35: 8411-22.
33. LEE YJ, EUN JR: Cilostazol decreases ethanol-mediated TNF-alpha expression in RAW264.7 murine macrophage and in liver from binge drinking mice. *Korean J Physiol Pharmacol* 2012; 16: 131-8.
34. JUNG WK, LEE DY, PARK C *et al*.: Cilostazol is anti-inflammatory in BV2 microglial cells by inactivating nuclear factor-kappaB and inhibiting mitogen-activated protein kinases. *Br J Pharmacol* 2010; 159: 1274-85.