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Rebamipide inhibits tumor necrosis factor-α-induced interleukin-8 expression by suppressing the NF-κB signal pathway in human umbilical vein endothelial cells

Jung-Yoon Choe · Ki-Yeun Park · Seung-Jin Lee · Sung-Hoon Park · Seong-Kyu Kim

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Abstract
Objective This study was designed to identify the inhibitory effect of rebamipide on tumor necrosis factor-α (TNF-α)-induced interleukin-8 (IL-8) production and nuclear factor-κB (NF-κB) activation in human umbilical vein endothelial cells (HUVECs).
Methods After stimulation with TNF-α, HUVECs were treated with rebamipide in a dose-dependent manner. The viability of HUVECs was assessed using methylthiazol tetrazolium assay after 24 h incubation with rebamipide. TNF-α-induced IL-8 expression was determined by enzyme-linked immunosorbent assay (ELISA) and quantitative real-time polymerase chain reaction (RT–PCR). TNF-α-induced IκB-α phosphorylation and translocation of NF-κB p65 subunit into nucleus in endothelial cells were assessed using immunoblot analysis.
Results We found that rebamipide decreased the expression of IL-8 in the dose-dependent manner under the treatment with TNF-α 10 ng/ml. TNF-α-induced degradation of IκB-α at 15 min was maximally observed and rebamipide (2 mM) inhibited TNF-α-induced phosphorylation of IκB-α in the cytoplasm of endothelial cells by western blot analysis. Rebamipide also suppressed TNF-α-stimulated NF-κB p65 nuclear translocation.
Conclusion Rebamipide suppresses TNF-α-induced IL-8 production through (1) inhibition of IκB-α phosphorylation in the cytoplasm and (2) blockage of NF-κB p65 protein transport into the nucleus. We suggest that the anti-inflammatory effect of rebamipide is related to the down-regulation of IL-8 expression that is important in endothelial inflammation.

Keywords Rebamipide · Interleukin-8 · Tumor necrosis factor-α · Nuclear factor-κB · Human umbilical vein endothelial cell

Introduction

Recruitment and infiltration of leukocytes to injury sites are a hallmark of inflammation. Regulatory mechanisms for leukocyte recruitment to the vascular wall, including leukocyte adhesion and transmigration, have been well characterized. A number of adhesion molecules involved in binding between leukocytes and endothelial cells have been characterized, including vascular endothelial cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule (ICAM-1), CD11a/CD18 and several selectin molecules, which are known to be involved in the pathogenesis of diverse vascular diseases [1]. In addition, enhanced local production of chemokines, known as leukocyte chemotactic polypeptides of 8–14 kD, may also mediate the inflammatory process. Various chemokines, including interleukin-8 (IL-8), monocyte chemoattractant

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The authors J.-Y. Choe and K.-Y. Park contributed equally to this manuscript.

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J.-Y. Choe · S.-H. Park · S.-K. Kim (✉)
Department of Internal Medicine, Catholic University of Daegu School of Medicine, 3056-6 Daemyung 4-Dong, Namgu, Daegu 705-718, South Korea
e-mail: kimsk714@cu.ac.kr

J.-Y. Choe · K.-Y. Park · S.-J. Lee · S.-H. Park · S.-K. Kim
Arthritis and Autoimmunity Research Center, Catholic University of Daegu School of Medicine, Daegu, South Korea

protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α) and interferon-inducible protein 10, are thought to foster leukocyte recruitment in inflammation and atherosclerosis [2].

IL-8 [alternatively known as CXC ligand-8 (CXCL-8)] is a prototypic human chemokine produced by several different types of cells, including vascular endothelial cells, T lymphocytes, and monocytes [3–5]. IL-8 is rarely released in non-stimulated cells but is markedly induced in response to external inflammatory stimuli including bacteria, oxidative stress, lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α) and IL-1 [4–7]. IL-8 gene expression can also be regulated by mechanisms including de-repression of the gene promoter, gene expression through the activation of nuclear factor-κB (NF-κB) and JNK mitogen-activated protein kinases (MARK), and post-transcriptional stabilization of IL-8 by p38 MARK [8].

Rebamipide (2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid) has been shown to have potent anti-inflammatory effects for gastric mucosal injury by diverse pathogenic stimuli including *H. pylori* infection, non-steroidal anti-inflammatory drugs and necrotizing agents [9]. The pharmacological action of rebamipide consists of the reduction of oxygen free radicals including nitric oxide synthetase, attenuation of neutrophil activity, and inhibition of production of pro-inflammatory cytokines and chemokines including TNF-α, IL-6 and IL-8. Among the latter inflammatory molecules, it has been well established that increased IL-8 production in response to stimuli such as *H. pylori* infection play an important role in the activation of neutrophils in gastric mucosal inflammation [10]. NF-κB is a well-known transcription factor involved in IL-8 gene expression. The inhibition of NF-κB signal pathways in gastric epithelial cell lines including AGS and Kato III cells has been shown to decrease production of IL-8 [11, 12]. They also showed inhibitory effects of rebamipide on IL-8 production by suppressing activation of NF-κB transcription factor. However, no study has yet examined the regulatory effects of rebamipide on IL-8 expression in endothelial cells.

In the present study, we investigated whether rebamipide regulates TNF-α-induced IL-8 expression through the NF-κB signal pathway in human umbilical vein endothelial cells (HUVECs).

Materials and methods

Cell culture and treatment

The HUVECs were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in MSCGM Bullet Kit (Lonza, Gaithersburg, Maryland, USA) and in EGM-2 Bullet Kit (Lonza, Gaithersburg, Maryland, USA) at 37°C in 5% CO_2 humidified air. In all experiments, cells were stimulated with 10 ng/ml of human recombinant TNF-α (R&D System, Minneapolis, MN, USA) for 24 h. Rebamipide was dissolved in 10 mM NaOH solution at a concentration of 10 mM for cellular experiments. Cells were used between the 3–6 passages.

Cytotoxicity assay

To assess cell viability experiment, cells were seeded at 2×10^4 cells/well in 96-well culture plate and were treated with different doses of rebamipide and incubated for 24 h. After various treatments, the medium was removed and the cells were incubated with 0.5 mg/ml of methylthiazol tetrazolium (MTT) solution. After incubation for 4 h at 37°C and 5% CO_2, the supernatant was removed and DMSO was added. After incubation at room temperature for 10 min, the formation of formazan was measured at 540 nm with a microplate reader.

Determination of IL-8 level using enzyme-linked immunosorbent assay (ELISA)

IL-8 level was determined by the ELISA method (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. In brief, 96-well microtiter plates were coated with monoclonal anti-human IL-8 antibody. After blocking, the plates were incubated with samples overnight. The plates were washed three times and biotinylated anti-IL-8 antibody was added. After 2 h of incubation, the plates were washed three times, and streptavidin-HRP conjugate was added, and the plates were incubated for 20 min at room temperature. The plates were then washed again and the substrate was added. Finally, 50 μL of stop solution was added and the plates were gently tapped to ensure thorough mixing. The optical density of each well was determined using a microplate reader set to 450 nm.

Quantitative real-time polymerase chain reaction (RT–PCR)

Total RNA was extracted from the cells using Trizol reagent (Gibco BRL, Grand Island, NY, USA) according to the manufacturer's protocol. Quantitative RT–PCR was performed using the Mini Option TM Real-time PCR system (Bio-Rad, Hercules, CA, USA) with DyNAmo SYBR Green qPCR kit (FINNZYMES, Espoo, Finland) according to the manufacturers' instructions. Reactions started with an initial heating at 95°C for 15 min, 40 cycles of denaturation at 95°C for 10 s, annealing/extension at 60°C for 60 s. Primers for IL-8 forward: 5′-TGCCAAGGAGTGCTAAAG-3′, reverse:

5′-CTCCACAACCCTCTGCAC-3′ and β-actin forward: 5′-CTGGAACGGTGAAGGTGACA-3′ reverse: 5′-AA GGGACTTCCTGTAACAATGCA-3′ were synthesized (Bionics, Seoul, Korea). Real-time PCR data were analysed with delta Ct method and the amount of IL-8 was calculated relative to the amount of β-actin as control.

Western blot analyses

For preparation of the cytosolic and nuclear extracts, cells were washed with PBS, lysed in buffer A (10 mM HEPES, pH8.0, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.1% Nonidet P40, 0.5 mM phenylmethylsulfonyl fluoride), incubated on ice for 5 min and then centrifuged at 12,000 rpm for 1 min. The cytosolic extract (supernatant) was collected and stored at −80°C. For the nuclear extract, the cell pellet was resuspended in buffer B [20 mM HEPES (pH 8.0), 20% Glycerol, 100 mM KCl, 100 mM NaCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride]. After incubation on ice for 15 min and centrifugation at 12,000 rpm for 5 min, the supernatant was collected and the nuclear protein extract was obtained.

Protein extracts were electrophoresed on 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (BIO-RAD, CA, USA), which was blocked with 5% milk for 1 h, followed by incubation with the primary antibody at 4°C overnight. The primary antibodies used were polyclonal rabbit anti-human IκB (1:1,000), polyclonal rabbit anti-human phosphor-IκB (1:1,000) and NF-κB rabbit polyclonal antibody (1:1,000). A goat anti-rabbit IgG conjugated secondary antibody was diluted at a ratio of 1:2,000 for 1 h. Immunoreactive proteins were identified with the ELC western blot detection system kit (Amersham, Braunschweig, Germany).

Immunofluorescent analysis for NF-κB p65

HUVECs were seeded in chambered tissue culture slides at a density of 1×10^5 cells incubated overnight. After treatment with serum-free medium pretreated with rebamipide 1 mM for 24 h and TNF-α (10 ng/ml) for 15 min, cells were fixed 10% paraformaldehyde for 10 min at room temperature. Cells were then permeabilized in 0.1% Tween 20 in PBS for 5 min. Cells were incubated with rabbit polyclonal NF-κB p65 antibody (Santa Cruz Biotechnology, 1:100 dilution) for 1 h at room temperature. After PBS washing, the secondary antibody Texas red (Invitrogen, CA, USA) and Hoechst 33342 were applied for 15 min at room temperature. Slides were mounted using VECTASHIELD mounting medium (VECTOR Laboratories, CA, USA) and photographed using a fluorescence microscope.

Statistical analysis

All data were described as mean ± standard deviation. Statistical analysis was performed using the Student's t test. A p value less than 0.05 was considered statistically significant between two experimental groups.

Results

Cell viability assay of rebamipide-treated HUVECs

Analysis for HUVECs viability incubated with rebamipid at various dosages for 24 h was performed using MTT assay (Fig. 1). HUVECs were sustained without marked viability changes from 0.05 to 10 mM of rebamipide. Cell viability was significantly reduced at 50 and 100 mM dosages of rebamipide compared with the group treated with a dosage of 1 mM ($p < 0.05$ and $p < 0.01$, respectively).

TNF-α-induced IL-8 production in HUVECs

We investigated the production of IL-8 in HUVECs using the ELISA method after treatment with 3, 10, and 30 ng/ml of TNF-α for 24 h (Fig. 2a). All dosages of TNF-α significantly increased IL-8 production compared with basal groups ($p < 0.001$ of all dosage groups). The effect of exposure times of 10 ng/ml TNF-α on IL-8 production was also examined at base, 2, 4, 6, 8, 12, and 24 h. Figure 2b shows that IL-8 production increased with TNF-α treatment over 24 h in a time-dependent manner.

Rebamipide inhibits TNF-α-induced IL-8 production in HUVECs

To investigate the effect of rebamipide on IL-8 production, HUVECs were treated with various concentrations of

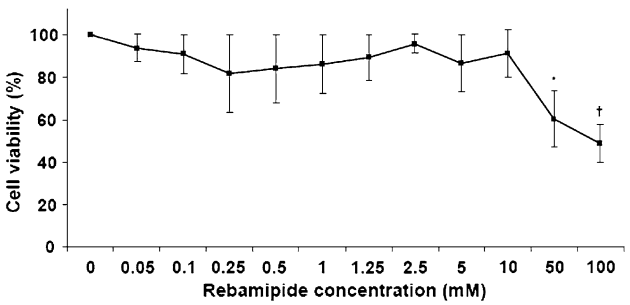

Fig. 1 Cell viability assay in the HUVECs incubated with rebamipide. HUVECs were treated with rebamipide for 24 h at various dosages. At groups treated with 50 and 100 mM of rebamipide, cell viability was significantly decreased compared with that with 10 mM (*$p < 0.05$, $^{\dagger}p < 0.01$, respectively). Data were determined in three independent experiments

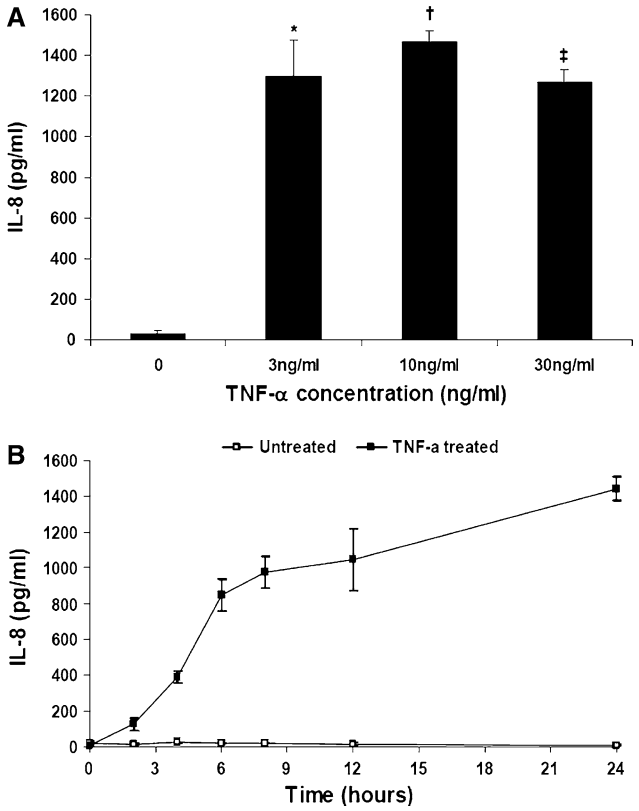

Fig. 2 IL-8 production at untreated and TNF-α stimulated cells. **a** TNF-α stimulation in HUVECs for 24 h induced significantly increased production of IL-8 at all dosages of TNF-α (*$p < 0.001$, †$p < 0.001$, ‡$p < 0.001$). **b** IL-8 production was markedly increased after stimulation of 10 ng/ml TNF-α in a time-dependant manner. Data were determined in three independent experiments

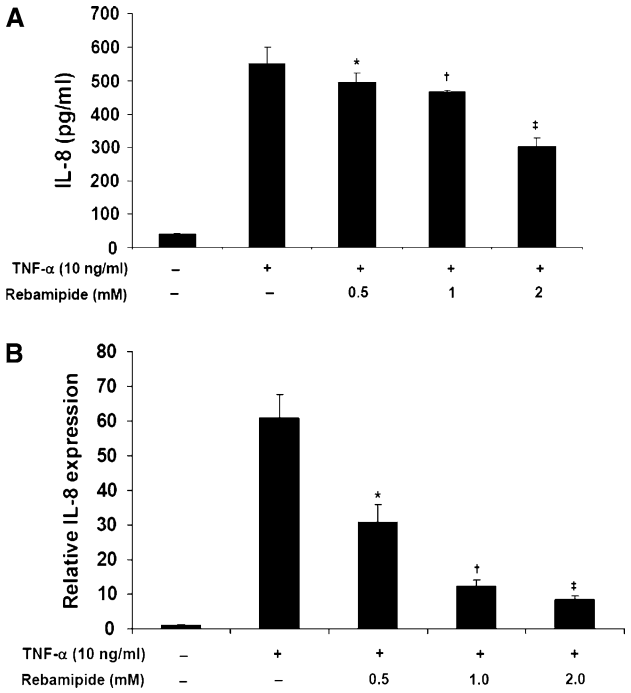

Fig. 3 Rebamipide inhibits IL-8 production in TNF-α stimulated HUVECs. **a** IL-8 production was significantly attenuated by pretreatment for 4 h with TNF-α and then cultured with 1.0 and 2.0 mM rebamipide for 24 h (†$p < 0.05$, ‡$p < 0.001$, respectively), but not of 0.5 mM (*$p > 0.05$). The level of IL-8 production was measured by the ELISA. **b** After pretreatment with TNF-α for 6 h, rebamipide significantly decreased IL-8 mRNA expression in 0.5 mM of, 1 mM of, and 2 mM of rebamipide using quantitative RT–PCR (*$p < 0.001$ of 0.5 mM, †$p < 0.001$ of 1 mM, ‡$p < 0.001$ of 2 mM, respectively). Data were determined in three independent experiments

rebamipide under stimulation of 10 ng/ml TNF-α for 4 h. Rebamipide inhibited TNF-α-induced IL-8 production in a dose-dependent manner (Fig. 3a). When compared with TNF-α alone, 1.0 mM and 2.0 mM of rebamipide statistically reduced IL-8 production ($p < 0.05$, $p < 0.001$, respectively), but no significant difference between TNF-α alone and 0.5 mM rebamipide-treated HUVECs was noted.

We examined IL-8 mRNA expression through RT–PCR analysis. HUVECs were pretreated with 10 mg/ml TNF-α for 6 h and were then stimulated with rebamipide for 18 h at various dosages. Figure 3b demonstrated that IL-8 m RNA expression was significantly decreased at dosages of 0.5, 1.0, and 2.0 mM of rebamipide using quantitative RT–PCR ($p < 0.001$ of 0.5 mM, $p < 0.001$ of 1 mM, and $p < 0.01$ of 2 mM, respectively).

Rebamipide inhibits TNF-α-induced IκB-α phosphorylation and degradation

NF-κB is retained in its inactive form in the cytoplasm by binding with the inhibitor of NF-κB, IκB-α. Various cytokine inducers such as TNF-α, IL-1β, and IL-17 activate IκB kinase which phosphorylates the IκB/NF-κB complex in the cytoplasm. Phosphorylated IκB separates from NF-κB and is processed via ubiquination and degradation in proteosomes.

We investigated expression of IκB-α and phospho-IκB-α proteins after stimulation with TNF-α for 15 min in cytosolic extracts from the cytoplasm of HUVECs (Fig. 4). TNF-α stimulation decreased IκB-α expression and augmented phosphor-IκB-α expression. In addition, rebamipide treatment for 2 h induced IκB-α expression ($p < 0.001$) but decreased phosphor-IκB-α expression ($p < 0.01$). This finding suggests that rebamipide blocks dissociation of IκB-α from the IκB/NF-κB complex by inhibition of the phosphorylation of IκB-α.

Rebamipide inhibits TNF-α-induced translocation of p65 subunit of NF-κB to nucleus

NF-κB dissociated from the IκB/NF-κB complex enters into the nucleus, resulting in stimulation of the transcription of various cytokines, chemokines, and adhesion molecules. We assessed the expression of NF-κB p65

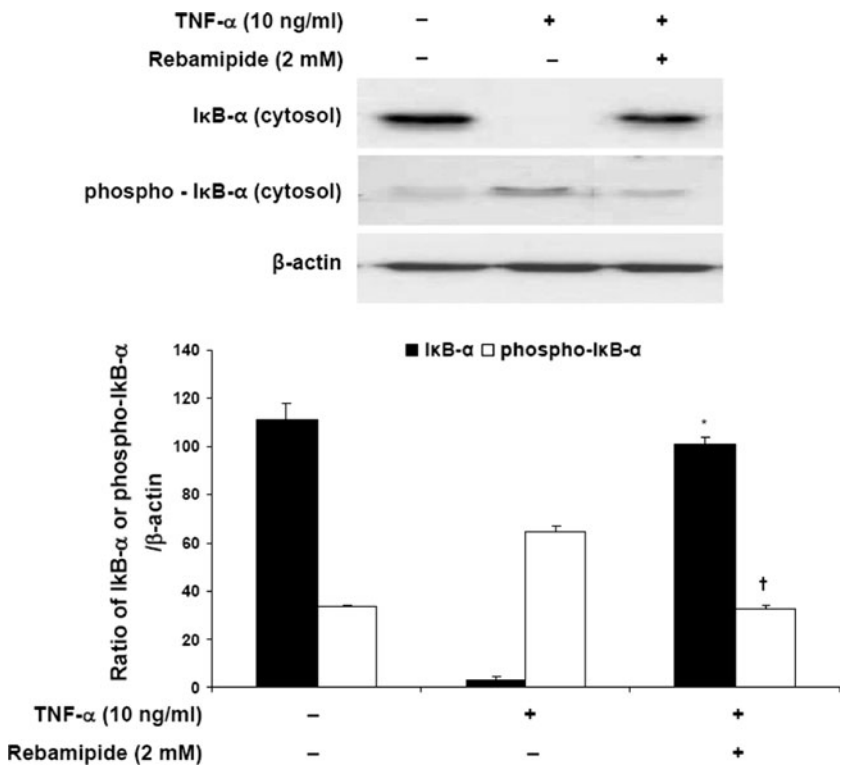

Fig. 4 Rebamipide blocks phosphorylation of IkB-α in NF-κB signal pathway. HUVEC were pretreated with TNF-α for 15 min and then cultured with 2 mM rebamipide for 2 h. TNF-α stimulation induced more prominent phosphor-IκB-α protein than IκB-α at the analysis of the cytoplasm in HUVECs, but rebamipide reversed activation of these subunits of NF-κB transcription factor. (*$p < 0.001$, compared to IκB-α treated with only TNF-α and $^{\dagger}p < 0.01$, compared to phospho-IκB-α treated with only TNF-α). Data were determined in three independent experiments

(RelA), one member of the NF-κB family, in both the cytoplasm and the nucleus of HUVECs. Western blotting assay demonstrated that TNF-α stimulation induced NF-κB p65 subunit expression in the nucleus rather than the cytoplasm ($p < 0.01$), whereas rebamipide inhibited NF-κB p65 expression in the nucleus ($p < 0.001$) (Fig. 5a).

We compared nuclear translocation of NF-κB p65 with TNF-α alone and in combination with rebamipide. Immunofluorescent staining study showed that exposure to TNF-α caused increased positive NF-κB p65 antibody staining in the nucleus, but rebamipide significantly attenuated nuclear staining for NF-κB p65 (Fig. 5b). The results suggest that rebamipide inhibits translocation of the TNF-α-induced NF-κB p65 subunit into the nucleus.

Discussion

Leukocyte recruitment to injured tissues and organs is an important characteristic of inflammation. Leukocyte recruitment is initiated by endothelial cells activated via diverse stimuli including pro-inflammatory cytokines, infection by bacteria or viruses, and hemodynamic disturbances [13]. Leukocyte adhesion to and transmigration though the vascular endothelium involves a number of adhesion molecules and chemokines. IL-8, the prototypic chemokine secreted by endothelial cells, T lymphocytes, and monocytes, mediates neutrophil attraction to and activation at inflammatory sites [3–5]. IL-8 is not detected in resting cells, but production is increased in response to a variety of external stimuli including oxidative stress, TNF-α, IL-1, and LPS [4–7]. In the present study, we observed IL-8 production in HUVECs under TNF-α stimulation as compared to untreated cell groups. We examined the initial increase in IL-8 over 2 h of TNF-α stimulation and the gradual, persistent increase in IL-8 over 24 h of TNF-α stimulation. TNF-α stimulation at dosages of 3, 10, and 30 ng/ml was found to significantly increase IL-8 expression in HUVECs. Our results confirm that IL-8 production can be induced by TNF-α stimulation, affirming previous results by Lakshminarayanan et al. [6].

Rebamipide has been well established as an anti-inflammatory drug for gastric mucosal injury via action mechanisms including reduction of oxygen free radicals, reduction of neutrophil activation, and suppression of pro-inflammatory cytokines in animal and human studies [9]. Recently Moon et al. [14] reported that rebamipide plays a role as agonist for Ca^{2+}-mobilization and as antagonist for cholecystokinin with amylase-releasing action. Gastric epithelial cell studies have demonstrated a close relationship between IL-8 production and gastric injury by *H. pylori*-infection or ceramide among sphingolipid metabolites [11, 12]. Their results demonstrated that therapeutic mechanisms of rebamipide for gastric injury are related to inhibition of lipid peroxidation, *H. pylori*-induced NF-κB activation and IL-8 production. Recent

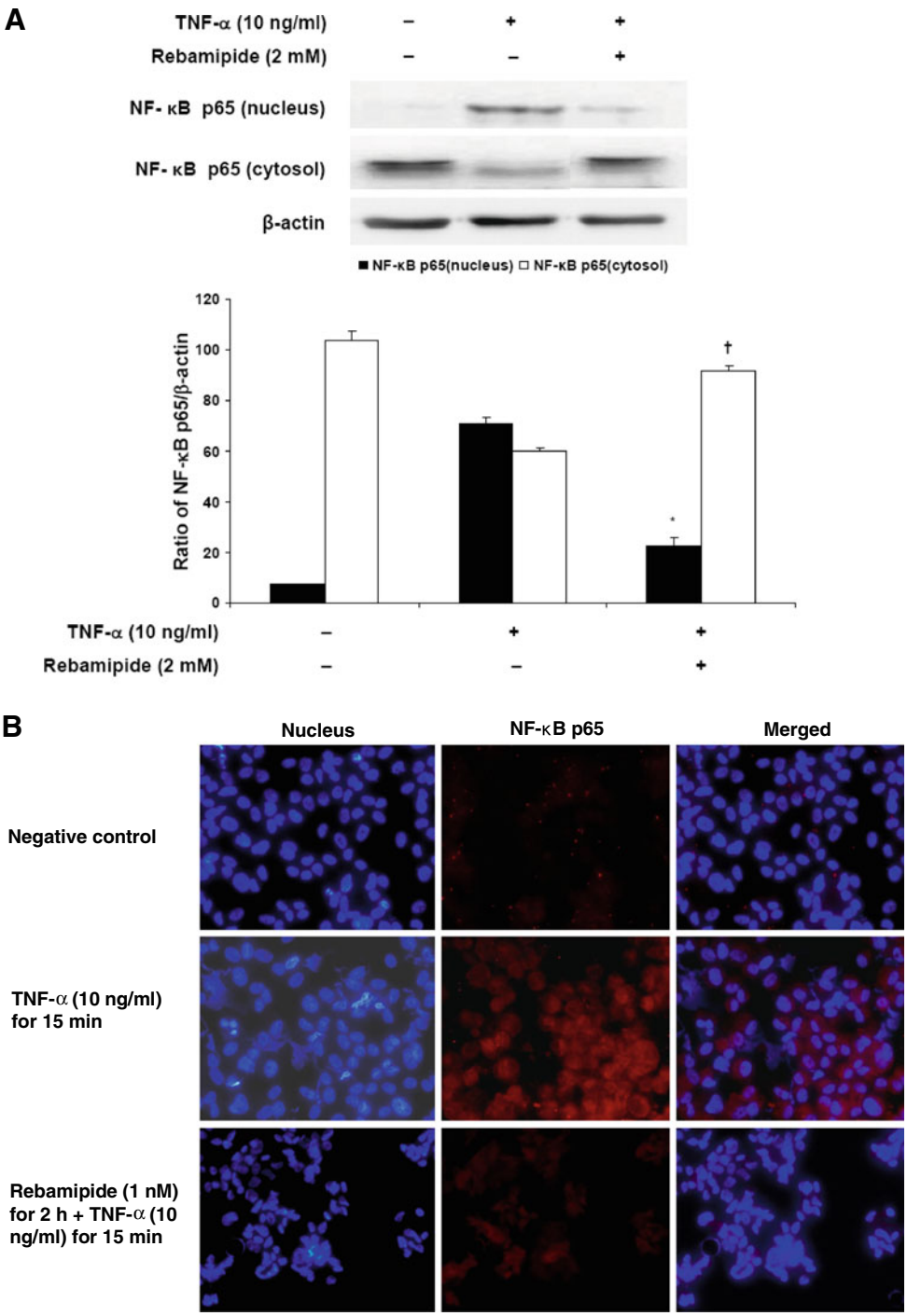

Fig. 5 Rebamipide inhibits translocation of NF-κB into the nucleus. The rebamipide was stimulated with TNF-α for 15 min after treatment. **a** NF-κB p65 subunit in the nucleus by stimulation of TNF-α was attenuated by rebamipide treatment (*$p < 0.001$, compared to NF-κB treated with only TNF-α). In addition, rebamipide increased cytosolic NF-κB p65 expression (†$p < 0.01$, compared to NF-κB treated with only TNF-α). **b** Immunofluorescent staining analysis for NF-κB p65 expression was performed in HUVECs. Cells were pretreated with TNF-α for 15 min and then incubated with rebamipide for 2 h. The analysis showed no expression of NF-κB p65 under these conditions without stimulation of both TNF-α and rebamipide. Staining of NF-κB p65 antibody was prominent in the nucleus after only TNF-α (10 ng/ml) stimulation without rebamipide. However, rebamipide (1 mM) under treatment of TNF-α (10 ng/ml) attenuated nuclear staining for NF-κB p65 in HUVECs. Data were determined in three independent experiments

data have also been published regarding the inhibitory effects of rebamipide on neutrophil adhesion [15, 16]. Kim et al. [16] reported rebamipide's anti-inflammatory effects against *H. pylori*-infected gastric mucosal inflammation through inhibition of neutrophil adhesion to endothelial cells, inhibition of CD11b adhesion molecules on neutrophil and inhibition of IL-8 production in gastric epithelial cells. The same study group also investigated the inhibitory mechanism of rebamipide on neutrophil adhesion to endothelial cells. They demonstrated that rebamipide inhibits hypoxia/reoxygenation-induced NF-κB activation and inhibits surface adhesion molecules such as P-selectin and E-selectin in HUVECs [15]. In the present study, we sought to identify the mechanism of rebamipide inhibition of TNF-α-induced IL-8 production. Our results demonstrate rebamipide attenuation of IL-8 production in 10 ng/ml TNF-α-stimulated endothelial cells as measured by ELISA and RT–PCR methods. We also identified the

inhibitory effects of rebamipide on IL-8 production via the blockage of NF-κB translocation into the nucleus of endothelial cells.

IL-8 gene expression is regulated via the activation of NF-κB transcription factor and MARK pathways including JNK and p38 MARKs [8]. NF-κB transcription factor is usually in dimeric form composed of two subunits, p65 (rel A) and p50 subunits. Other subunits include rel, rel B, v-rel, and p52. NF-κB is retained in the cytoplasm and bound to IκB which inhibits NF-κB translocation into the nucleus. When cells are activated, phosphorylation of IκB induces its release from the NF-κB/IκB complex and allows NF-κB translocation into the nucleus and binding to the IL-8 gene promoter region. NF-κB is required for the regulation of inflammatory and immune genes including TNF-α, IL-1β, IL-8, MIP-1α, VCAM-1, and ICAM-1, which play a critical role in normal host defense and cell apoptosis, and a pathological role in chronic inflammatory disease [17]. Therefore, NF-κB may be a potent therapeutic target for anti-inflammatory therapy. Several studies have demonstrated the efficacy of targeting NF-κB transcription factor for reducing inflammation. Shono et al. [5] showed that anti-sense NF-κB oligonucleotide inhibits hydrogen peroxide-induced IL-8 production at the transcriptional level in cultured human vascular endothelial cells. In a study using rheumatoid arthritis synovial fibroblasts, adenoviral-mediated gene delivery of a nondegradable IκBα decreased IL-1-induced IL-8 production, mediated by inhibition of NF-κB activation [7]. In our study, we identified the mechanism of rebamipide for inhibition of IL-8 production in TNF-α-stimulated endothelial cells. IκB-α has a critical role in the inhibition of NF-κB translocation into the nucleus. Thus, the presence of both naive IκBα and phospho-IκB-α forms before and after TNF-α stimulation in the cytoplasm of endothelial cells were identified. We found that IκBα expression was prominent without any treatment, and that TNF-α induced phosphorylation of IκBα. We then identified that rebamipide inhibits phosphorylation of IκB-α in the endothelial cytoplasm using western blot. Finally, we investigated translocation of NF-κB into the nucleus using western blotting and immunofluorescent analysis. Our result showed expression of NF-κB p65 subunit by TNF-α stimulation was significantly reduced after treatment with rebamipide. These findings suggest that rebamipide inhibits IL-8 production via inhibition of IκB-α phosphorylation and translocation of the NF-κB transcription factor.

In addition to the therapeutic effect of rebamipide on gastric mucosal injury, clinical applications of rebamipide in various clinical fields have been studied. Sjogren syndrome is an inflammatory autoimmune disease characterized by lymphocyte infiltrations and tissue damage of exocrine glands including lacrimal and salivary glands, resulting in diverse clinical features such as dry mouth and dry eyes [18]. The main pathogenic mechanism is thought to occur between epithelial cells and autoreactive $CD4^+$ T cells, leading to the production of inflammatory cytokines and autoantibodies by B cells [18]. Kohashi et al. [19] demonstrated rebamipide suppressed activation of $CD4^+$ cells and inflammatory cytokines in NFS/*sld* mice model. Rebamipide demonstrated an inhibitory effect on infiltration of inflammatory cells, especially granulocytes, to inflammation sites through an experimental colitis rat model using dextran sulfate sodium [20]. Our study also confirmed that rebamipide suppressed enhanced migration of HUVECs stimulated by TNF-α (data not shown, Supplement file). In addition, one clinical study of rebamipide therapy against oral ulcers in Behcet's disease, a type of chronic inflammatory vasculitis, found a beneficial therapeutic effect for recurrent aphthous oral ulcer [21]. Recently, Bang et al. [22] also illustrated that rebamipide increased the therapeutic efficacy of colchicine via suppression of NADPH oxidase in a Behcet's disease mouse model. After examining this evidence, we concluded that rebamipide may be an important therapeutic drug for vascular inflammation and other inflammatory diseases. The results from our study for the molecular mechanism between IL-8 production and NF-κB involvement using HUVECs may be applicable to diverse clinical diseases involved in vascular inflammation, such as Behcet's disease.

In conclusion, we found that rebamipide inhibits IL-8 production via blockage of TNF-α-induced NF-κB activation. In this study, we showed that inhibition of NF-κB activation is related to blocking of IκB-α phosphorylation in endothelial cells.

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