Department of Surgery[1], College of Medicine, Kyung Hee University; Medical Science Research Institute[2], Kyung Hee University Medical Center, Seoul, Republic of Korea

Activin suppresses the inflammatory response of TNF-α-stimulated human umbilical vein endothelial cells

Hyunmin Ko[1], Young Il Kim[2], Hyung Joon Ahn[1,*]

Received February 7, 2022, accepted March 13, 2022

**Corresponding author: Prof. Hyung Joon Ahn, Department of Surgery, College of Medicine, Kyung Hee University, 23 Kyung Hee Dae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea*
whipple@khu.ac.kr

Pharmazie 77: 152-156 (2022) *doi: 10.1691/ph.2022.2330*

Activins belong to the transforming growth factor (TGF)-β superfamily and are involved in the regulation of homeostasis, proliferation, differentiation, and inflammation. In the present study, we examined the mechanism by which activin regulates the transcription of tumor necrosis factor-α (TNF-α)-stimulated cytokines, chemokines, toll-like receptors (TLRs), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) in human umbilical vein endothelial cells (HUVECs), and the involvement of the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. Cell viability was analyzed using MTS/PES solution, mRNA expression was measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and protein expression was measured by immunoblotting. TNF-α increased the mRNA expression of cytokines (IL-1β and IL-6), chemokines (IL-8 and MCP-1), and TLR2, as well as the mRNA and protein expression of iNOS and COX-2. Activin decreased TNF-α-induced cytokine, chemokine, and TLR mRNA expression as well as TNF-α-induced iNOS and COX-2 mRNA and protein expression. In addition, activin suppressed the phosphorylation of NF-κB p65 in TNF-α-stimulated HUVECs and reduced TNF-α-induced phosphorylation of AKT, JNK, ERK, and p38 MAPK. Our results demonstrate that the anti-inflammatory effects of activin are mediated by inflammatory response genes through the inhibition of NF-κB and AKT/JNK/MAPK signaling.

1. Introduction

Activin, a member of the transforming growth factor-β (TGF-β) superfamily, was originally identified based on its ability to stimulate the release of follicle-stimulating hormone from cultured pituitary gonadotropes (Sosnowski et al. 2000; Abel et al. 2001). Activins are formed by homo- or heterodimeric proteins of the βA/βA-subunit (activin A), βB/βB-subunits (activin B), or βA/βB-subunits (activin AB) (Vale et al. 1986). The biological signaling of activins is mediated by receptor complexes that consist of two different activin serine/threonine kinase receptors (ActR): type I (ActR I) and type II (ActR II) (Mathews 1994). Activin is involved in the regulation of homeostasis, development, proliferation, apoptosis, differentiation, and inflammation in a diverse range of cellular systems (Namwanje and Brown 2016).

Inflammation is a vital component of the immune response to injury and infection. It signals the immune system to heal and repair damaged tissue, as well as defend the host against foreign invaders, such as viruses and bacteria, and it is coordinated through a complex network of cytokine signaling pathways (Ranjbar et al. 2019). Tumor necrosis factor-α (TNF-α) is a critical inflammatory factor that causes interactions between vascular endothelial cells and invading monocytes (Libby et al. 1986). TNF-α also plays a crucial role in the atherosclerotic inflammatory cascade by upregulating the expression of adhesion molecules, endothelial cell activation, and vascular remodeling (Ragab et al. 2015). Furthermore, treatment with TNF-α blockers reduces the number of infiltrating synovial granulocytes and macrophages and decreases the expression of the chemokines IL-8 and monocyte chemotactic protein-1 (Taylor et al. 2000). TNF-α is a pleiotropic cytokine produced by many cell types in the body. TNF-α is a powerful proinflammatory agent that regulates many aspects of macrophage function. TNF-α plays an important role in the proliferation, apoptosis, and differentiation of macrophages. This function of TNF-α seems to be dependent on the phosphatidylinositol 3-kinase, AKT, and MEK/ERK signaling pathways (Lee et al. 2001).

Toll-like receptors (TLRs) play important roles in microbial detection. Pathogen recognition by innate immune cells is regulated by pattern recognition receptors. It transduces signals through a variety of pattern recognition receptors, including TLRs, nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and retinoic acid-inducible gene (RIG)-1-like receptors (Takeuchi and Akira. 2010; Kvarnhammar et al. 2011). It also induces the activation of nuclear factor-κB (NF-κB), leading to the production of several proinflammatory cytokines and chemokines (Lawrence. 2009; Liu et al. 2017). Various genes, such as those encoding inflammatory cytokines and TNF-α, regulate signaling through TLRs in macrophages and other immune competitors (Covacu et al. 2009). Understanding the immune system and the regulatory function of the inflammatory response during the mechanistic action of NF-κB suggests new possibilities for inflammatory response and immunotherapy after attempting to develop therapeutic agents using TNF-α inhibitors (Hayden and Ghosh 2014). However, research on whether TNF-α-activated human umbilical vein endothelial cells (HUVECs) can be regulated by the anti-inflammatory factor activin A is inadequate.

We examined the mechanism by which activin regulates the transcription of TNF-α-stimulated TLRs, cytokines, inducible NOS (iNOS), and cyclooxygenase-2 (COX-2) in HUVECs, and our results indicate that it involves the NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways.

2. Investigations and results

2.1. Effects of activin A on HUVECs

HUVECs were treated with various concentrations of activin A (0-50 ng/mL) for 24 h. Cell proliferation was determined using

an MTT assay. Activin A at a concentration of 50 ng/mL reduced cell growth (Fig. 1A). HUVECs were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h before undergoing exposure to TNF-α for 20 h. Cells were observed using an inverted microscope. After undergoing TNF-α treatment, cell morphology was not changed by activin A but was activated by TNF-α (Fig. 1B).

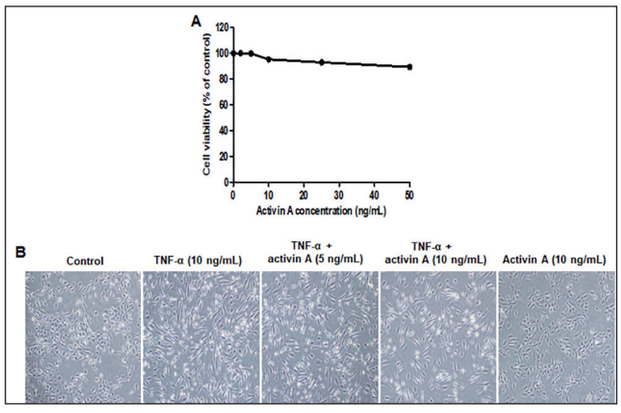

Fig. 1: Effects of TNF-α and activin on human umbilical vein endothelial cell (HUVEC) proliferation. Cells were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h before undergoing exposure to TNF-α (10 ng/mL) for 20 h. (A) Cell viability was determined using the MTS/PES assay. The data are expressed as the mean ± standard deviation of triplicate samples. Cell morphology of HUVECs under conditions of TNF-α treatment. (B) Cells were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h before undergoing exposure to TNF-α (10 ng/mL) for 24 h. The cell morphology was observed under an inverted microscope (magnification, ×100).

2.2. Activin inhibited TNF-α-activated cytokine, chemokine, and TLR mRNA expression in HUVECs

We evaluated whether activin affects the mRNA transcription of inflammatory cytokines. HUVECs were treated with TNF-α and activin, as described in the Experimental section. The mRNA expression levels of cytokines (IL-1β and IL-6) and chemokines (IL-8 and MCP-1) were determined by qPCR. TNF-α significantly increased cytokine and chemokine. mRNA expression compared with those of the control. Activin A suppressed TNF-α-induced cytokine (Fig. 2A and 2B) and chemokine (Fig. 2C and 2D) mRNA expression. TNF-α increased TLR2 mRNA expression compared with the control, whereas TLR4 was not activated. Activin A suppressed TNF-α-induced TLR2 mRNA expression (Fig. 2E and 2F).

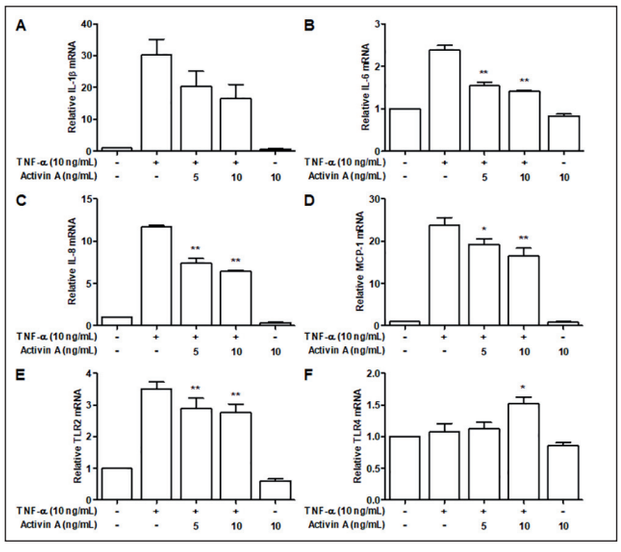

Fig. 2: Effects of activin on the TNF-α-induced cytokine, chemokine, and TLR mRNA expression levels in human umbilical vein endothelial cells. Cells were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h before undergoing exposure to TNF-α (10 ng/mL) for 20 h, and the mRNA expression levels were measured by qPCR. The crossing point of IL-1β (A), IL-6 (B), IL-8 (C), MCP-1 (D), TLR2 (E), and TLR4 (F) with β-actin was entered into the formula, $2^{-(\text{target gene}-\beta\text{ actin})}$, and the relative amounts were quantified. The data are expressed as the mean ± standard deviation of three independent samples. *p < 0.05 and **p < 0.01 compared with TNF-α stimulation alone.

2.3. Activin inhibited TNF-α-induced iNOS and COX-2 mRNA and protein expression in HUVECs

To examine the anti-inflammatory activity of activin, we examined the effects of activin on iNOS and COX-2 mRNA and protein expression in TNF-α-induced HUVECs. HUVECs were treated with TNF-α and activin, as described in the Methods section. mRNA and protein expression levels were measured by qPCR and immunoblot analysis. TNF-α increased iNOS and COX-2 mRNA and protein expression compared with those of the control. Activin suppressed TNF-α-induced iNOS (Fig. 3A and 3C) and COX-2 (Fig. 3B and 3D) mRNA and protein expression.

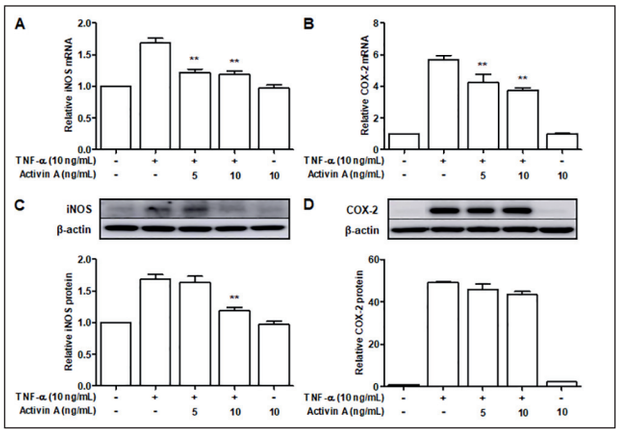

Fig. 3: Effects of activin on the TNF-α-induced iNOS and COX-2 mRNA and protein expression in human umbilical vein endothelial cells. Cells were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h before undergoing exposure to TNF-α (10 ng/mL) for 20 h. The mRNA expression levels were measured by qPCR. The crossing point of iNOS (A) and COX-2 (B) with β-actin was entered into the formula, $2^{-(\text{target gene}-\beta\text{ actin})}$, and the relative amounts were quantified. Protein expression was determined by western blot analysis. Densitometry analyses are presented as the relative ratios of iNOS (C), COX-2 (D), and β-actin. The data are expressed as the mean ± standard deviation of three independent samples. **p < 0.01 compared with TNF-α stimulation alone.

2.4. Activin inhibited TNF-α-induced activation of the NF-κB, AKT, and MAPK signaling pathways in HUVECs

We evaluated the activation of signal transduction by activin in relation to inflammation. HUVECs were treated with TNF-α and activin, as described in the Experimental section. The protein expression levels of NF-κB, AKT, and MAPK (c-JNK, ERK, and p38) were determined using western blot analysis. TNF-α increased the phosphorylation of NF-κB, AKT and MAPK signaling compared with that of the control. Activin A inhibited TNF-α-induced phosphorylation of NF-κB (Fig. 4A and 4B), AKT (Fig. 4A and 4C), JNK (Fig. 4A and 4D), ERK (Fig. 4A and 4E) and p38 MAPK (Fig. 4A and 4F) signaling in HUVECs.

3. Discussion

Activin plays important physiological roles in cell differentiation and inflammation (Bloise et al. 2019) and can inhibit cell growth and induce apoptosis in many cancer cells (Chen et al. 2000, 2002). However, the effects of activin on HUVECs remain unclear. We investigated whether activin regulates the transcription of genes encoding cytokines, chemokines, TLRs, iNOS, and COX-2 and modulates the NF-κB, AKT and MAPK signaling pathways in TNF-α-stimulated HUVECs.

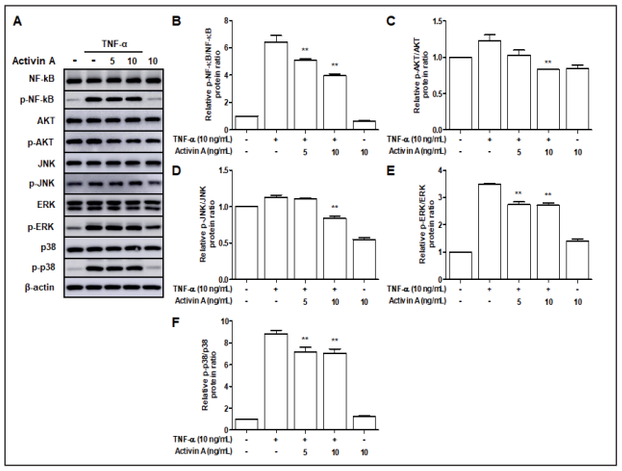

Fig. 4: Effects of activin on the TNF-α-induced phosphorylation of NF-κB p65, AKT, JNK, ERK, and p38 MAPK proteins in human umbilical vein endothelial cells. Cells were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h before undergoing exposure to TNF-α (10 ng/mL) for 20 h, and protein expression levels were determined by immunoblot analysis (A). Densitometry analyses are presented as the relative ratios of p-NF-κB p65/NF-κB p65 (B), p-AKT/AKT (C), p-JNK/JNK (D), p-ERK/ERK (E), p-p38 MAPK/p38 MAPK (F), and β-actin. The data are expressed as the mean ± standard deviation of three independent samples. **p < 0.01 compared with TNF-α stimulation alone.

When HUVECs were treated with different concentrations of activin A, toxicity was not observed at concentrations below 50 μg/mL Therefore, activin was determined to be at the appropriate concentration for stimulation in these experiments. However, TNF-α was applied at a concentration of 10 ng/mL in this study; therefore, this experiment was also stimulated at the same dose (Song et al. 2018; Nie et al. 2020). In the present study, we observed that the cell shape was activated in the TNF-α-treated HUVECs compared to the control cells and found that activin suppressed the TNF-α-induced mRNA expression of cytokines (IL-1β and IL-6) and chemokines (IL-8 and MCP-1). These results indicate that activin regulates the transcription of inflammatory cytokines and chemokines, suggesting that it acts as an anti-inflammatory agent in response to TNF-α in HUVECs. TNF-α increased adhesion and enhanced the inflammatory response in HUVECs. Apolipoprotein M and sphingosine-1-phosphate have been shown to attenuate TNF-α-induced injury and inflammatory responses in HUVECs (Liu and Tie 2019). Zafirlukast inhibits TNF-α-induced expression of cytokines (IL-1β, IL-6, and IL-8) in primary human aortic endothelial cells (Zhou et al. 2019). HUVECs showed increased mRNA expression levels of IL-1β, IL-6, TNF-α, and iNOS by lipopolysaccharide (LPS), which decreased after treatment with hyperoside (Wei et al. 2020).

The initial sensing of infectious microbes and other infections, which eventually activate the inflammatory response, is through an innate pattern recognition receptor, which includes TLRs, RIG-I-like receptors, and nucleotide-binding and oligomerization domain (NOD)-like receptors (Takeuchi and Akira 2010; Kvarnhammar et al. 2011). TLR expression has been reported in multiple tissues and cell types, including epithelial and endothelial cells (Grote et al. 2011). In the present study, we demonstrated that activin is regulated by TLR transcription in HUVECs. In the present study, activin inhibited the TNF-α-stimulated mRNA expression of TLR2 but did not affect the mRNA levels of TLR4. These results suggest that activin regulates inflammation at the transcription level in TLR2.

Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) and plays a role in the pathogenesis of several inflammatory diseases. NO is produced via the enzymatic action of NOS, for which both constitutive (cNOS) and inducible (iNOS) isoforms exist (Xue et al. 2018). HUVECs expressed iNOS during the primary culture, and iNOS induction was not related to LPS (Cristina de Assis et al. 2002). To determine whether activin could have a similar inhibitory action on HUVECs, we investigated its effects on NO in the presence of TNF-α. In the present study, we showed that activin inhibited iNOS mRNA and protein expression and blocked the stimulatory action of TNF-α on iNOS mRNA and protein expression. These findings suggest that activin inhibits iNOS at the gene transcription and translation levels in inflammation-activated HUVECs. In L929 cells, TNF-α significantly increased iNOS mRNA expression, whereas activin had no significant effect on iNOS mRNA expression. TNF-α promotes NO synthesis and secretion, whereas activin has no effect because it only plays a role in regulating NO secretion by TNF-α-activated cells (Jiang et al. 2020). We found that when HUVECs were treated with TNF-α, the expression of COX-2 increased. However, its expression was inhibited by activin, suggesting that activin regulates the level of gene transcription and translation of COX-2. TNF-α induces an increase in COX-2 expression in human NCI-H292 epithelial cells, but dexamethasone suppresses the mRNA and protein expression of COX-2 (Imseis et al. 1997). Triptolide inhibited COX-2 expression by post-transcriptional regulation of COX-2 mRNA in TNF-α-treated A549 cells (Sun et al. 2011). IL-35 suppressed the TNF-α-induced inflammatory response by inhibiting iNOS and COX-2 in peripheral blood monocyte-derived macrophages (Peng et al. 2019). Catechin inhibited TNF-α-induced gene expression of proinflammatory cytokines, including IL-1α, IL-1β, IL-6, and IL-12p35, and inflammatory enzymes, including iNOS and COX-2, in 3T3-L1 adipocytes (Cheng et al. 2019).

The NF-κB signaling pathway is activated by extracellular signaling factors and induces the expression of various proinflammatory genes, including cytokines and chemokines. Quercetin suppresses TNF-α-induced inflammation in HUVECs by inhibiting the NF-κB signaling pathway (Chen et al. 2020). Activated NF-κB acts as a transcription factor and induces the expression of several inflammatory genes, such as iNOS, TNF-α, IL-1β, and IL-6 (Kunnumakkara et al. 2018). MAPKs play an important regulatory role in the production of proinflammatory cytokines that induce inflammation. The MAPK family comprises three subfamilies: the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK subfamilies. MAPKs are involved in gene regulation by transducing extracellular signals into the nucleus (Moens et al. 2013). We found that activin inhibited the activation of TNF-α-induced NF-κB, AKT, and MAPK (JNK, ERK, and p38) signaling pathways in HUVECs. Carbocisteine effectively suppressed TNF-α-induced inflammation in A549 cells by inhibiting the NF-κB and ERK1/2 MAPK signaling pathways (Wang et al. 2016). Taken together, our results reveal that activin regulates anti-inflammatory effects via the modulation of MAPK phosphorylation and inactivation of NF-κB in TNF-α-activated HUVECs.

In conclusion, our results demonstrated that activin inhibited the TNF-α-induced expression of cytokines, chemokines, TLRs, iNOS, and COX-2 in HUVECs. Moreover, activin regulates anti-inflammatory effects through the modulation of AKT/JNK/MAPK phosphorylation and inactivation of NF-κB in TNF-α-activated HUVECs.

4. Experimental

4.1. Cell culture

HUVECs were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA) and were cultured in EBM-2 medium with growth supplement (Lonza, Walkersville, MD, USA) and antibiotics. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO^2 in 95% air. For treatment with TNF-α and activin A (R&D Systems, Minneapolis, MN, USA), HUVECs in serum-free medium were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h and then incubated with TNF-α (10 ng/mL) for 20 h.

4.2. TNF-α and activin A treatment

HUVECs in serum-free medium were pretreated with activin A (0, 5 and 10 ng/mL) for 2 h and then incubated with TNF-α (10 ng/mL) for 20 h. At each time point, total RNA and protein were isolated from the cultured HUVECs.

4.3. Cell proliferation assay

Cell proliferation was measured using CellTiter 96 Aqueous One Solution (Promega, Madison, WI, USA). Cells were seeded in 96-well plates at a density of 1 × 10^4 cells/well and incubated with TNF-α and activin A for 24 h. Cell viability was determined using a colorimetric assay with MTS/PES solution. Absorbance was measured at 492 nm with background subtraction at 650 nm. The cell morphology was observed under an inverted microscope (Olympus IX51, Tokyo, Japan, magnification, ×100).

4.4. RNA isolation and RT-qPCR

Total RNA was purified from the cultured cells using the RNeasy Mini Kit according to the manufacturer's protocol (Qiagen, Chatsworth, CA, USA). cDNA synthesis was performed with 1 µg of total RNA and transcribed to cDNA using a Revert Aid First Strand cDNA synthesis kit with Oligo (dT)[18] (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's protocol. The primer sequences used are listed in the Table. Quantitative PCR (qPCR) was performed on a StepOnePlus real-time PCR system with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA, USA). The PCR mixture consisted of 1 µL cDNA in a 20 µL reaction mixture containing 10 µL Power SYBR Green PCR Master Mix, 2 µL primers, and 7 µL PCR-grade water. The reactions were performed with a denaturation step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The crossing point of target genes with β-actin was calculated using the formula $2^{-(\text{target gene}-\beta\text{ actin})}$, and the relative amounts were quantified.

Table: Primers used for real-time PCR amplification.

Genes	Primer sequences	Product size (bp)	Annealing Temperature (°C)
IL-1β	5'-TGATGGCTTATTACAGTGGCAATG-3' 5'-GTAGTGGTGGTCGGAGATTCG-3'	140	60
IL-6	5'-GTGTTGCCTGCTGCCTTC-3' 5'-AGTGCCTCTTTGCTGCTTTC-3'	194	60
IL-8	5'-GACATACTCCAAACCTTTCCAC-3' 5'-CTTCTCCACAACCCTCTGC-3'	160	60
IL-10	5'-GAACCAAGACCCAGACATC-3' 5'-CATTCTTCACCTGCTCCAC-3'	137	60
MCP-1	5'-CAGCCAGATGCAATCAATGCC-3' 5'-TGGAATCCTGAACCCACTTCT-3'	190	60
TLR2	5'-TCTCCCATTTCCGTCTTTTT-3' 5'-GGTCTTGGTGTTCATTATCTTC-3'	125	60
TLR4	5'-GAAGCTGGTGGCTGTGGA-3' 5'-TGATGTAGAACCCGCAAG-3'	213	60
iNOS	5'-TGGATGCAACCCCATTGTC-3' 5'-CCCGCTGCCCCAGTTT-3'	59	60
COX-2	5'-CAAATCCTTGCTGTTCCCACCCAT-3' 5'-GTGCACTGTGTTTGGAGTGGGTTT-3	173	60
β-actin	5'-GCGAGAAGATGACCCAGATC-3' 5'-GGATAGCACAGCCTGGATAG-3'	77	60

4.5. Immunoblot analysis

Cells were washed with cold phosphate-buffered saline and lysed using lysis buffer containing 1 mM phenylmethylsulfonyl fluoride (Cell Signaling Technology, Boston, MA, USA). Protein concentration was determined using a BCA protein assay. Protein (10 µg) was fractionated on 10% SDS-PAGE and transferred onto a nitrocellulose membrane by electrophoresis. The membranes were blocked with 5% nonfat dry milk for 1 h at room temperature and then incubated overnight at 4°C with antibodies against iNOS (R&D Systems, Minneapolis, MN, USA), COX-2, NF-κB, AKT, JNK, ERK, p38 (Cell Signaling Technology), and β-actin (Sigma-Aldrich) antibodies (Cell Signaling Technology) at a 1:1000 dilution in Tris-buffered saline containing 0.05% Tween-20 (TBS-T) for 1 h. After washing with TBS-T for 1 h, the membranes were incubated with anti-rabbit and anti-mouse horseradish peroxidase-conjugated secondary antibodies diluted to 1:2500 (1:10000 for β-actin) in TBS-T for 1 h at room temperature. The membranes were subsequently washed with TBS-T for 1 h, and the proteins were developed using Amersham ECL Prime reagent (GE Healthcare Life Sciences, UK). The protein band densities were quantified using ImageJ software (NIH, USA).

4.6. Statistical analyses

Data from the statistical analysis are expressed as the mean±standard error. Data were compared using one-way analysis of variance and Tukey's post hoc test. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). *P < 0.05 and **P < 0.01 were considered to indicate statistical significance.

Acknowledgements: This work was supported by a grant from Kyung Hee University in 2019 (KHU-20192443) and the Korean Society of Vascular Surgery Lee Yong Gak-Astellas Research Fund (2019).

Conflicts of interest: None declared.

References

Abel MH, Wootton AN, Wilkins V, Huhtaniemi I, Knight PG, Charlton HM (2000) The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. Endocrinology 141: 1795–1803

Bloise E, Ciarmela P, Cruz CD, Luisi S, Petraglia F, Reis FM (2019) Activin A in mammalian physiology. Physiol Rev 99: 739–780

Chen T, Zhang X, Zhu G, Liu H, Chen J, Wang Y, He X (2020) Quercetin inhibits TNF-α induced HUVECs apoptosis and inflammation via downregulating NF-κB and AP-1 signaling pathway in vitro. Medicine (Baltimore) 99: e22241

Chen W, Woodruff TK, Mayo KE (2000) Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and smad proteins. Endocrinology 141: 1263–1272

Chen YG, Lui HM, Lin SL, Lee JM, Ying SY (2002) Regulation of cell proliferation, apoptosis, and carcinogenesis by activin. Exp Biol Med (Maywood) 227: 75–87

Cheng AW, Tan X, Sun JY, Gu CM, Liu C, Guo X (2019) Catechin attenuates TNF-α induced inflammatory response via AMPK-SIRT1 pathway in 3T3-L1 adipocytes. PLoS One 14: e0217090

Covacu R, Arvidsson L, Andersson A, Khademi M, Erlandsson-Harris H, Harris RA, Svensson MA, Olsson T, Brundin L (2009) TLR activation Induces TNF-α production from adult neural stem/progenitor cells. J Immunol 182: 6889–6895

Cristina de Assis M, Cristina Plotkowski M, Fierro IM, Barja-Fidalgo C, de Freitas MS (2002) Expression of inducible nitric oxide synthase in human umbilical vein endothelial cells during primary culture. Nitric Oxide 7: 254–261

Grote K, Schütt H, Schieffer B (2011) Toll-like receptors in angiogenesis. ScientificWorldJournal 11: 981–991

Hayden MS, Ghosh S (2014) Regulation of NF-κB by TNF family cytokines. Semin Immunol 26: 253–266

Imseis HM, Zimmerman PD, Samuels P, Kniss DA (1997) Tumour necrosis factor-α induces cyclo-oxygenase-2 gene expression in first trimester trophoblasts: suppression by glucocorticoids and NSAIDs. Placenta 18: 521–526

Jiang L, Liu B, Qi Y, Zhu L, Cui X, Liu Z (2020) Antagonistic effects of activin A and TNF-α on the activation of L929 fibroblast cells via Smad3-independent signaling. Sci Rep 10: 20623

Kunnumakkara AB, Sailo BL, Banik K, Harsha C, Prasad S, Gupta SC, Bharti AC, Aggarwal BB (2018) Chronic diseases, inflammation, and spices: how are they linked? J Transl Med 16: 14

Kvarnhammar AM, Petterson T, Cardell LO (2011) NOD-like receptors and RIG-I-like receptors in human eosinophils: activation by NOD1 and NOD2 agonists. Immunology 134: 314–325

Lawrence T (2009) The nuclear factor NF-κB pathway in inflammation. Cold Spring Harb Perspect Biol 1: a001651

Lee SE, Chung WJ, Kwak HB, Chung CH, Kwack KB, Lee ZH, Kim HH (2001) Tumor necrosis factor-α supports the survival of osteoclasts through the activation of Akt and ERK. J Biol Chem 276: 49343–49349

Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK, Dinarello CA (1986) Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. Am J Pathol 124: 179–185

Liu T, Zhang L, Joo D, Sun SC (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2: 17023

Liu Y, Tie L (2019) Apolipoprotein M and sphingosine-1-phosphate complex alleviates TNF-α-induced endothelial cell injury and inflammation through PI3K/AKT signaling pathway. BMC Cardiovasc Disord 19: 279

Mathews LS (1994) Activin receptors and cellular signaling by the receptor serine kinase family. Endocr Rev 15: 310–325

Moens U, Kostenko S, Sveinbjørnsson B (2013) The Role of mitogen-activated protein kinase-activated protein kinases (MAPKAPKs) in inflammation. Genes (Basel) 4: 101–133

Namwanje M, Brown CW (2016) Activins and inhibins: roles in development, physiology, and disease. Cold Spring Harb Perspect Biol 8: a021881

Nie N, Bai C, Song S, Zhang Y, Wang B, Li Z (2020) Bifidobacterium plays a protective role in TNF-α-induced inflammatory response in Caco-2 cell through NF-κB and p38MAPK pathways. Mol Cell Biochem 464: 83–91

Peng M, Qiang L, Xu Y, Li C, Li T, Wang J (2019) IL-35 ameliorates collagen-induced arthritis by promoting TNF-α-induced apoptosis of synovial fibroblasts and stimulating M2 macrophages polarization. FEBS J 286: 1972–1985

Ragab SM, Safan MA, Obeid OM, Sherief AS (2015) Lipoprotein-associated phospholipase A2 (Lp-PLA2) and tumor necrosis factor-alpha (TNF-α) and their relation to premature atherosclerosis in beta-thalassemia children. Hematology 20: 228–238

Ranjbar R, Shafiee M, Hesari A, Ferns GA, Ghasemi F, Avan A (2019) The potential therapeutic use of renin-angiotensin system inhibitors in the treatment of inflammatory diseases. J Cell Physiol 234: 2277–2295

Song HS, Kwon JE, Baek HJ, Kim CW, Jeon H, Ra JS, Lee HK, Kang SC (2018) Sorghum fermented by Aspergillus oryzae NK enhances inhibition of vascular inflammation in TNF-α-stimulated human aortic smooth muscle cells. Int J Vitam Nutr Res 88: 309–318

Sosnowski R, Mellon PL, Lawson MA (2000) Activation of translation in pituitary gonadotrope cells by gonadotropin-releasing hormone. Mol Endocrinol 14: 1811–1819

Sun L, Zhang S, Jiang Z, Huang X, Wang T, Huang X, Li H, Zhang L (2011) Triptolide inhibits COX-2 expression by regulating mRNA stability in TNF-α-treated A549 cells. Biochem Biophys Res Commun 416: 99–105

Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. Cell 140:805-820

Taylor PC, Peters AM, Paleolog E, Chapman PT, Elliott MJ, McCloskey R, Feldmann M, Maini RN (2000) Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor α blockade in patients with rheumatoid arthritis. Arthritis Rheum 43: 38–47

Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J (1986) Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature 321: 776–779

Wang W, Guan WJ, Huang RQ, Xie YQ, Zheng JP, Zhu SX, Chen M, Zhong NS (2016) Carbocisteine attenuates TNF-α-induced inflammation in human alveolar epithelial cells in vitro through suppressing NF-κB and ERK1/2 MAPK signaling pathways. Acta Pharmacol Sin 37: 629–636

Wei A, Xiao H, Xu G, Yu X, Guo J, Jing Z, Shi S, Song Y (2020) Hyperoside protects human umbilical vein endothelial cells against anticardiolipin antibody-induced injury by activating autophagy. Front Pharmacol 11: 762

Xue Q, Yan Y, Zhang R, Xiong H (2018) Regulation of iNOS on immune cells and its role in diseases. Int J Mol Sci 19: 3805

Zhou X, Caib, J, Liu W, Wu X, Gao C (2019) Cysteinyl leukotriene receptor type 1 (CysLT1R) antagonist zafirlukast protects against TNF-α-induced endothelial inflammation. Biomed Pharmacother 111: 452–459