Glutamine Inhibits TNF- α -induced Cytosolic Phospholipase A₂ Activation via Upregulation of MAPK Phosphatase-1

So Young Yoon*, Soo-Yeon Jeong* and Suhn-Young Im^{†,**}

*Department of Biological Sciences, College of Natural Sciences,
Chonnam National University, Gwangju 61186, Korea*

Tumor necrosis factor alpha (TNF- α) is a principal regulator of inflammation and immunity. The proinflammatory properties of TNF- α can be attributed to its ability to activate the enzyme cytosolic phospholipase A₂ (cPLA₂), which generates potent inflammatory lipid mediators, eicosanoids. L-glutamine (Gln) plays physiologically important roles in various metabolic processes. We have reported that Gln has a potent anti-inflammatory activity via rapid upregulation of mitogen-activated protein kinases (MAPKs) phosphatase (MKP)-1, which preferentially dephosphorylates the key pro-inflammatory enzymes, p38 MAPK and cytosolic phospholipase A₂ (cPLA₂). In this study, we have investigated whether Gln could inhibit TNF- α -induced cPLA₂ activation. Gln inhibited TNF- α -induced increases in cPLA₂ phosphorylation in the lungs and blood levels of the cPLA₂ metabolites, leukotriene B₄ (LTB₄) (lipoxygenase metabolite) and prostaglandin E₂ (PGE₂) (cyclooxygenase metabolite). TNF- α increased p38 and cPLA₂ phosphorylation and blood levels of LTB₄ and PGE₂, which were blocked by the p38 inhibitor SB202190. Gln inhibited TNF- α -induced p38 and cPLA₂ phosphorylation and production of the cPLA₂ metabolites. Such inhibitory activity of Gln was no longer observed in MKP-1 small interfering RNA-pretreated animals. Our data indicate that Gln inhibited TNF- α -induced cPLA₂ phosphorylation through MKP-1 induction/p38 inhibition, and suggest that the utility of Gln in inflammatory diseases in which TNF- α plays a major role in their pathogenesis.

Key Words: Glutamine, TNF- α , cPLA₂, p38 MAPK, MKP-1

INTRODUCTION

TNF- α is produced mainly by monocytes and macrophages, and is a key cytokine regulating inflammation and immunity (Taylor et al., 2004). Many studies have showed that TNF- α is a principal therapeutic target for many inflammatory diseases (Kalliolias and Ivashkiv, 2016). TNF- α activates the pro-inflammatory enzyme, cytosolic phospholipase A₂ (cPLA₂) through increasing cPLA₂ phosphorylation

and causing the translocation of cPLA₂ from perinuclear regions to the plasma membrane (Hoeck et al., 1993; Hirabayashi and Shimizu, 2000; Sapirstein and Bonventre, 2000), which can explain the proinflammatory properties of TNF- α . TNF- α -uses a serial pathway involving ROS/mitogen-activated protein kinase (MAPK)s/NF- κ B/p300 (Lee et al., 2013; Lin et al., 2016) for cPLA₂ activation. cPLA₂ is involved in the production of potent lipid inflammatory mediator, eicosanoids such as platelet-activating factor (PAF), leukotrienes (LTs) (5-lipoxygenase products),

Received: October 25, 2021 / Revised: December 3, 2021 / Accepted: December 6, 2021

*Researcher, **Professor.

[†]Corresponding author: Suhn-Young Im. Department of Biological Sciences, College of Natural Sciences, Chonnam National University, Gwangju 61186, Korea.

Tel: +82-62-530-3414, Fax: +82-62-530-3409, e-mail: syim@chonnam.ac.kr

©The Korean Society for Biomedical Laboratory Sciences. All rights reserved.

©This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

and prostaglandins (PGs) and thromboxane (cyclooxygenase products) via releasing arachidonic acid (for an overview, see Dennis et al., 2011). Studies using cPLA₂ knock-out animals which display reduction in inflammatory and allergic responses indicate the key role of cPLA₂ in inflammatory, cardiovascular and neurological diseases (Leslie, 2015).

L-glutamine (Gln), a non-essential amino acid, is the most abundant amino acid in human body and used as an energy fuel in most cells (Fox et al., 1996; Encarnacion et al., 1998). Gln is an important molecule in the synthesis of peptides, nucleotide bases, neurotransmitters, and glutathione (Albrecht et al., 2010; Amores-Sanchez and Medina, 1999). Critically ill patients undergo Gln's alteration, which leads to muscle proteolysis activation, insulin resistance, and increased liver gluconeogenesis (Griffiths et al., 1997). Gln supplementation has been shown to decrease infectious complications and shortens hospitalization (Griffiths, 2003). We have reported that Gln exerts beneficial effects against several experimental inflammatory diseases (Kim et al., 2006; Ko et al., 2008; Ayush et al., 2013; Im et al., 2018). Such effect of Gln was due to its ability to induce MAPK phosphatase-1 (MKP-1) protein (Ko et al., 2009), which dephosphorylates p38 and JNK (Franklin and Kraft, 1997; Hammer et al., 2006). As a result, MKP-1 inactivates cPLA₂ by dephosphorylating p38, as cPLA₂ is one of p38 substrate (Su and Karin, 1996). Although we have reported MKP-1 upregulation as the anti-inflammatory mechanism of Gln in many experimental inflammatory diseases, it is unknown whether the same mechanism will be operated in Gln inhibition of the pro-inflammatory property of TNF. Therefore, in this study, we have investigated whether Gln could inhibit TNF- α -induced cPLA₂ phosphorylation via MKP-1 induction and p38 inhibition.

MATERIALS AND METHODS

Animals

Specific pathogen-free female C57BL/6 mice were obtained from Orient Bio (Seongnam, Gyeonggi, Korea) and housed in clean, pathogen-free rooms in an environment with controlled temperature (23 °C), humidity (55%), and a 12 hr light/dark cycle. All mice were used at 6~7 weeks of

age. All experiments were conducted in accordance with the guidelines of the Chonnam National University Institutional Animal Care and Use Committee (Approval No. CNU IACUC-YB-2018-05). We included 4 mice/group/time point /experiment.

Reagents

L-Gln (biotechnology performance certified, G-8540) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Gln was dissolved in sterilized distilled water to reach 4%, the saturated concentration at room temperature. Gln (750 mg /kg) was administered to animals via intraperitoneally (i.p.). Control mice received vehicle only. Recombinant mouse TNF- α was purchased from R&D System (Minneapolis, MN, USA). The p38 MAPK inhibitor SB202190 was obtained from Calbiochem (San Diego, CA, USA). SB202190 (5 mg /kg i.p.) was injected twice 48 hr and 24 hr (Lee et al., 2012; Kim et al., 2021) before TNF- α (25 μ g/kg) injection (Jia et al., 2013). Antibodies against phospho-p38, phospho-cPLA₂, and MKP-1 were purchased from Cell Signaling Technology (Danvers, MA, USA).

Cell culture

Murine alveolar macrophage cells, MH-S (ATCC CRL-2019), were maintained in RPMI 1640 containing 2 mM of Gln (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum and 1% antibiotics (Invitrogen, Carlsbad, CA). The cells were maintained at 37 °C in a humidified atmosphere at 5% CO₂. Cell passages, between 4~20, used throughout this study.

Measurement of LTB₄ and PGE₂

Serum levels of LTB₄ and PGE₂ were quantified using competitive enzyme-linked immunosorbent assay (ELISA) according to the protocol of the manufacturers from Cayman Chemical Company (Ann Arbor, MI, USA) and R&D System (Minneapolis, MN, USA), respectively.

Western blot analysis

Mice were sacrificed by cervical dislocation and the lungs were collected, frozen immediately in liquid nitrogen and were stored at -70 °C until analysis. The lung specimens

Fig. 1. Gln inhibits TNF- α -induced cPLA₂ phosphorylation and metabolites. A, Mice were injected i.v. with TNF- α (25 μ g/kg) and the lungs were obtained at the indicated times. B and C, SB202190 (5 mg/kg) was administered i.p twice (-2 and -1 days) before TNF- α injection, and the lungs were collected 15 min after TNF- α injection. Serum was prepared 2 hr after TNF- α injection. Representative immunoblots of phosphorylated form (p) of p38 and cPLA₂ in lung tissues (A and B). C, Data represent mean $\pm$ SE. of three independent experiments ($n = 4$ /group/time point). * $P < 0.01$ vs. normal control group; # $P < 0.01$ vs. TNF- α -treated group.

and whole cell extracts were homogenized in PhosphoSafe Extraction Reagent (Novagen Merck, Darmstadt, Germany) with phenylmethylsulfonyl fluoride protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA). Western blot analysis was performed as described previously (Jeong and Im, 2019).

Small interfering RNA interference

Small interfering RNA (siRNA) strands for MKP-1 and controls were obtained from Santa Cruz Biotechnology (mRNA accession # NM: 013642, Santa Cruz, CA, USA). The target sequences are as follow; Duplex 1 sense strand: GGUUCAACGAGGCUAUUGA, Duplex 2 sense strand: CGAGGCUAUUGACUUCAUA, Duplex 3 sense strand: GCAAGACAUUUGCUGAACU. *In vivo* delivery of siRNA was performed using *In vivo*-jet polyethyleneimine (PEI; Polyplus-transfection, Illkirch, France), according to the instructions of the manufacturer. In brief, MKP-1 siRNA and PEI dissolved in 5% glucose were mixed in a volume of 200 μ L for i.v. injection at room temperature for 20 min, and the mixture was administered 24 hr before Gln administration. The mixture containing control siRNA and PEI dissolved in 5% glucose without siRNA were used as controls. The mixture almost completely inhibits the target molecule expression (Ko et al., 2009; Ayush et al., 2013).

Statistical analysis

Data are expressed as means $\pm$ SE. Statistical significance was determined via one-way analysis of variance (Stat-View; Abacus Concepts Inc., Berkeley, CA, USA). A value of $P < 0.05$ was regarded as statistically significant. All experiments were conducted at least twice. Reproducible results were obtained and representative data are, therefore, provided in the figures.

RESULTS

p38 MAPK plays a key role in TNF- α -mediated phosphorylation of cPLA₂

We first examined how p38 MAPK regulates TNF- α -induced cPLA₂ phosphorylation in the lungs. Administration of TNF- α resulted in phosphorylations of p38 MAPK and cPLA₂ at 5~20 min (Fig. 1A). The p38 inhibitor SB202190 inhibited TNF- α -induced cPLA₂ phosphorylation (Fig. 1B) and increases in blood levels of the two cPLA₂ metabolites, LTB4 and PGE2 (Fig. 1C), indicating that p38 is required for cPLA₂ activation in response to TNF- α .

Fig. 2. Gln inhibits TNF- α -induced phosphorylation of p38 and cPLA₂ phosphorylation as well as the production of LTB4 and PGE2. A and B, Gln (750 mg/kg, i.p.) was given 10 min after TNF- α injection. A, The lungs were obtained at the indicated times. B, Serum was prepared 2 hr after TNF- α injection. C and D, MH-S cells (2×10^6) were treated with Gln (40 mM) 10 min after TNF- α treatment. Cell lysate was prepared at the indicated times. Representative immunoblots of p-p38 and p-cPLA₂ using lung tissues (A) and cell lysates (C). GAPDH served as a loading control. B and D, Data represent mean $\pm$ SE. of three independent experiments ($n = 4$ /group/time point). * $P < 0.05$ vs. normal control group; # $P < 0.01$ vs. normal control group; + $P < 0.01$ vs. TNF- α -treated group.

Gln inhibits TNF- α -mediated phosphorylation of p38 and cPLA₂ and production of cPLA₂ metabolites

Gln nearly completely inhibited TNF- α -induced phosphorylation of p38 and cPLA₂ at 15 and 20 min when Gln was given at 10 min post TNF- α injection (Fig. 2A). The blood levels of the LTB4 and PGE2 in TNF- α -injected mice were also inhibited by Gln (Fig. 2B). We also examined the Gln's effect using the murine alveolar macrophage cell line MH-S. Addition of TNF- α increased the phosphorylation of p38 and cPLA₂, which were dephosphorylated by adding Gln 10 min after TNF- α stimulation (Fig. 2C). Gln also inhibited TNF- α -induced increases in LTB4 and PGE2 production (Fig. 2D), as seen in *in vivo* study.

MKP-1 induction/p38 inhibition is involved in Gln inhibition of TNF- α -mediated phosphorylation of cPLA₂

MKP-1 appeared from 15 min in response to TNF- α in the lungs (Fig. 3A). Gln administration at 10 min post-TNF- α resulted in not only early appearance but also potentiation of MKP-1 upregulation (Fig. 3B).

We examined the involvement of MKP-1 in Gln inhibition of TNF- α -induced cPLA₂ phosphorylation using MKP-1 siRNA. Gln again upregulated MKP-1 and inhibited p38 and cPLA₂ phosphorylation, and the effects of Gln were no longer observed in MKP-1 siRNA-, but not control siRNA-, treated mice (Fig. 3C). Furthermore, administration of MKP-1 siRNA, but not control siRNA, abolished the Gln-induced inhibition of LTB4 and PGE2 production (Fig. 3D). These data indicate that Gln deactivates TNF- α -induced cPLA₂ activation through MKP-1 upregulation.

Fig. 3. Gln potentiated TNF- α -induced MKP-1 induction and MKP-1 siRNA abrogates Gln inhibitions of TNF- α -induced cPLA₂ phosphorylation and production of LTb4 and PGE2. A-D, Gln was given 10 min after TNF- α injection. Lungs were removed 20 min (C) and serum was prepared at 2 hr (D) after TNF- α injection. Representative immunoblots of MKP-1 (A-C) and p-p38, p-cPLA₂ (C) in lung tissues. D, Data represent mean $\pm$ SE. of three independent experiments ($n = 4$ /group/time point). * $P < 0.01$ vs. normal control group; # $P < 0.01$ vs. TNF- α -treated group; + $P < 0.01$ vs. TNF- α + Gln-treated group.

DISCUSSION

Our previous reports have shown that Gln acts as a MKP-1 inducer, which deactivates not only p38 and JNK MAPKs, but also cPLA₂ by dephosphorylating them in lung tissues in many disease models (Kim et al., 2006; Ko et al., 2009). Therefore, we here demonstrated that Gln, in addition to the beneficial effects against inflammatory disease, also exerts an anti-inflammatory action against a proinflammatory cytokine itself through the same mechanism. In this study, our observation that 1) Gln administration resulted in earlier and stronger upregulation of MKP-1 and dephosphorylation of p38 and cPLA₂ in TNF- α -injected mice, and 2) MKP-1 siRNA abolished such effect of Gln, indicated that such effect of Gln was attributed to the early upregulation of MKP-1. Regarding the administration time and concentration of Gln *in vivo*, we have reported that Gln induced MKP-1 upregulation within 5 min after administration and the optimum concentration was 750 mg/kg (Ko et al., 2009, Ayush et al., 2013; Im et al., 2018).

MKPs are subfamilies within a larger group of dual-specificity protein phosphatases which dephosphorylate MAPK. MKP-1 has been reported as an ERK-specific phosphatase (Sun et al., 1993; Misra-Press et al., 1995), but dephosphorylate and inactivate both p38 and JNK MAPKs later (Franklin and Kraft, 1997; Chi et al., 2006; Hammer et al., 2006; Zhao et al., 2006). Given that both p38 and cPLA₂ are key enzymes involved in inflammation, MKP-1 is regarded as a negative regulator of inflammatory responses. Some stress stimuli can induce MKP-1 through transcriptional (Li et al., 2001; Wang et al., 2007) and post-transcriptional mechanisms (Brondello et al., 1999; Lin and Yang, 2006). These include oxidative stress and heat shock (Keyse and Emslie, 1992), anti-cancer drugs (Chattopadhyay et al., 2006; Wang et al., 2006) and UV light (Franklin et al., 1998). As a post-transcriptional mechanism, Brondello et al. (Brondello et al., 1999) reported that ERK MAPK phosphorylates MKP-1 on two carboxyl-terminal serine residues - serine 359 and serine 364, resulting in the stabilization of MKP-1 by preventing proteosomal degradation. We have reported

that Gln increase of ERK activity via activation of the Ca^{2+} /Ras/c-Raf/MEK (ERK cascade) pathway as a post-transcriptional mechanism (Ayush et al., 2016).

TNF- α is importantly involved in the pathogenesis of many important inflammatory diseases such as rheumatoid arthritis, Crohn's disease, psoriatic arthritis, juvenile idiopathic arthritis, psoriasis, and ankylosing spondylitis (Kallioli and Ivashkiv, 2016). Although the action of TNF- α has not been fully elucidated in the context, the ability of TNF- α to activate cPLA₂ can explain its proinflammatory properties because cPLA₂ is involved in the generation of the potent pro-inflammatory lipid mediator, eicosanoids. These molecules are importantly associated with the pathogenesis of rheumatoid arthritis (Feuerherm et al., 2019), Crohn's disease (Rosengarten et al., 2016), psoriasis (Omland et al., 2017), and other autoimmune diseases (Marusic et al., 2008; Yang et al., 2014).

The approved anti-TNF agents have been widely used in the treatment of TNF-associated diseases (Monaco et al., 2015). However, it has been reported that the clinical use of TNF has been turned out to have several limitations, such as 1) low rates of disease remission, 2) increase in common and opportunistic infections, i.e., reactivation of latent tuberculosis, and 3) induction of autoantibodies, lupus-like symptoms, and increased risk for specific malignancies, such as lymphomas (Smith and Kauffman, 2009; Deepak et al., 2013; Feldmann and Maini, 2015).

In summary, we found that Gln successfully inhibited TNF- α -induced cPLA₂ phosphorylation. Given that supportive nutritional Gln therapy is safe (Wischmeyer et al., 2001; Novak et al., 2002), Gln may provide a therapeutic regimen to many inflammatory diseases in which TNF- α plays an important role in their pathogenesis.

ACKNOWLEDGEMENT

This study was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2016R1D1A3B03934457).

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article

was reported.

REFERENCES

- Albrecht J, Sidoryk-Wegrzynowicz M, Zielinska M, Aschner M. Roles of glutamine in neurotransmission. *Neuron Glia Biol.* 2010. 6: 263-276.
- Amores-Sanchez MI, Medina MA. Glutamine, as a precursor of glutathione, and oxidative stress. *Mol Genet Metab.* 1999. 67: 100-105.
- Ayush O, Jin ZW, Kim HK, Shin Y, Im SY, Lee HK. Glutamine up-regulates MAPK phosphatase-1 induction via activation of $\text{Ca}^{2+} \rightarrow$ ERK cascade pathway. *Biochem Biophys Rep.* 2016. 7: 10-19.
- Ayush O, Lee CH, Kim HK, Im SY, Cho BH, Lee HK. Glutamine suppresses dinitrofluorobenzene-induced contact dermatitis by deactivating p38 mitogen-activated protein kinase via induction of MAPK phosphatase-1. *J Invest Dermatol.* 2013. 133: 723-731.
- Brondello JM, Pouyssegur J, McKenzie FR. Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. *Science.* 1999. 286: 2514-2517.
- Chattopadhyay S, Machado-Pinilla R, Manguan-Garcia C, et al. MKP1/CL100 controls tumor growth and sensitivity to cisplatin in non-small-cell lung cancer. *Oncogene.* 2006. 25: 3335-3345.
- Chi H, Barry SP, Roth RJ, et al. Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. *Proc Natl Acad Sci USA.* 2006. 103: 2274-2279.
- Deepak P, Sifuentes H, Sherid MD, Stobaugh D, Sadozai Y, Ehrenpreis ED. T-cell non-Hodgkin's lymphomas reported to the FDA AERS with tumor necrosis factor-alpha (TNF- α) inhibitors: results of the REFURBISH study. *Am J Gastroenterol.* 2013. 108: 99-105.
- Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G. Phospholipase A₂ enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem Rev.* 2011. 111: 6130-6185.
- Encarnacion S, Calderon J, Gelbard AS, Cooper AJ, Mora J. Glutamine biosynthesis and the utilization of succinate and glutamine by *Rhizobium etli* and *Sinorhizobium meliloti*. *Microbiology.* 1998. 144: 2629-2638.
- Feldmann M, Maini RN. Perspectives from masters in rheumatology

- and autoimmunity: can we get closer to a cure for rheumatoid arthritis? *Arthritis Rheumatol.* 2015. 67: 2283-2291.
- Feuerherm AJ, Dennis EA, Johansen B. Cytosolic group IVA phospholipase A₂ inhibitors, AVX001 and AVX002, ameliorate collagen-induced arthritis. *Arthritis Res Ther.* 2019. 21: 29-41.
- Fox RE, Hopkins IB, Cabacungan ET, Tildon JT. The role of glutamine and other alternate substrates as energy sources in the fetal rat lung type II cell. *Pediatr Res.* 1996. 40: 135-141.
- Franklin CC, Kraft AS. Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. *J Biol Chem.* 1997. 272: 16917-16923.
- Franklin CC, Srikanth S, Kraft AS. Conditional expression of mitogen-activated protein kinase phosphatase-1, MKP-1, is cytoprotective against UV-induced apoptosis. *Proc Natl Acad Sci USA.* 1998. 95: 3014-3019.
- Griffiths RD. Nutrition support in critically ill septic patients. *Curr Opin Clin Nutr Metab Care.* 2003. 6: 203-210.
- Griffiths RD, Jones C, Palmer TE. Six-month outcome of critically ill patients given glutamine-supplemented parenteral nutrition. *Nutrition.* 1997. 13: 295-302.
- Hammer M, Mages J, Dietrich H, et al. Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. *J Exp Med.* 2006. 203: 15-20.
- Hirabayashi T, Shimizu T. Localization and regulation of cytosolic phospholipase A₂. *Biochim Biophys Acta.* 2000. 1488: 124-138.
- Hoeck WG, Ramesha CS, Chang DJ, Fan N, Heller RA. Cytoplasmic phospholipase A₂ activity and gene expression are stimulated by tumor necrosis factor: dexamethasone blocks the induced synthesis. *Proc Natl Acad Sci USA.* 1993. 90: 4475-4479.
- Im YN, Jeong SY, Youm JY, Lee HK, Im SY. L-glutamine attenuates DSS-induced Colitis via induction of MAPK phosphatase-1. *Nutrients.* 2018. 10: 288-297.
- Jia Z, Babu PV, Si H, et al. Genistein inhibits TNF- α -induced endothelial inflammation through the protein kinase pathway A and improves vascular inflammation in C57BL/6 mice. *Int J Cardiol.* 2013. 168: 2637-2645.
- Jeong SY, Im SY. Estrogen induces CK2 α activation via generation of reactive oxygen species. *Biomed Sci Letters.* 2019. 25: 23-31.
- Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol.* 2016. 12: 49-62.
- Keyse SM, Emslie EA. Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. *Nature.* 1992. 359: 644-647.
- Kim JM, Im YN, Chung YJ, et al. Glutamine deficiency shifts the asthmatic state toward neutrophilic airway inflammation. *Allergy.* 2021. Oct 3. doi: 10.1111/all.15121. Online ahead of print.
- Kim YS, Kim GY, Kim JH, et al. Glutamine inhibits lipopolysaccharide-induced cytoplasmic phospholipase A₂ activation and protects against endotoxin shock in mouse. *Shock.* 2006. 25: 290-294.
- Ko HM, Kang NI, Kim YS, et al. Glutamine preferentially inhibits T-helper type 2 cell-mediated airway inflammation and late airway hyperresponsiveness through the inhibition of cytosolic phospholipase A₂ activity in a murine asthma model. *Clin Exp Allergy.* 2008. 38: 357-364.
- Ko HM, Oh SH, Bang HS, et al. Glutamine protects mice from lethal endotoxic shock via a rapid induction of MAPK phosphatase-1. *J Immunol.* 2009. 182: 7957-7962.
- Lee CH, Kim HK, Kim JM, et al. Glutamine suppresses airway neutrophilia by blocking cytosolic phospholipase A(2) via an induction of MAPK phosphatase-1. *J Immunol.* 2012. 189: 5139-5146.
- Lee IT, Lin CC, Cheng SE, Hsiao LD, Hsiao YC, Yang CM. TNF- α induces cytosolic phospholipase A₂ expression in human lung epithelial cells via JNK1/2- and p38 MAPK-dependent AP-1 activation. *PLoS One.* 2013. 8: e72783-72793.
- Leslie CC. Cytosolic phospholipase A₂: physiological function and role in disease. *J Lipid Res.* 2015. 56: 1386-1402.
- Li J, Gorospe M, Hutter D, Barnes J, Keyse SM, Liu Y. Transcriptional induction of MKP-1 in response to stress is associated with histone H3 phosphorylation-acetylation. *Mol Cell Biol.* 2001. 21: 8213-8224.
- Lin CC, Lin WN, Cho RL, Wang CY, Hsiao LD, Yang CM. TNF- α -induced cPLA₂ expression via NADPH oxidase/reactive oxygen species-dependent NF- κ B cascade on human pulmonary alveolar epithelial cells. *Front Pharmacol.* 2016. 7: 447-462.
- Lin YW, Yang JL. Cooperation of ERK and SCFSkp2 for MKP-1 destruction provides a positive feedback regulation of proliferating signaling. *J Biol Chem.* 2006. 281: 915-926.
- Marusic S, Thakker P, Pelker JW, et al. Blockade of cytosolic phospholipase A₂ α prevents experimental autoimmune encephalomyelitis and diminishes development of Th1 and

- Th17 responses. *J Neuroimmunol*. 2008. 204: 29-37.
- Misra-Press A, Rim CS, Yao H, Roberson MS, Stork PJ. A novel mitogen-activated protein kinase phosphatase: structure, expression, and regulation. *J Biol Chem*. 1995. 270: 14587-14596.
- Monaco C, Nanchahal J, Taylor P, Feldmann M. Anti-TNF therapy: past, present and future. *Int Immunol*. 2015. 27: 55-62.
- Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementation in serious illness: a systematic review of the evidence. *Crit Care Med*. 2002. 30: 2022-2029.
- Omland SH, Habicht A, Damsbo P, Wilms J, Johansen B, Gniadecki RJ. A randomized, double-blind, placebo-controlled, dose-escalation first-in-man study (phase 0) to assess the safety and efficacy of topical cytosolic phospholipase A₂ inhibitor, AVX001, in patients with mild to moderate plaque psoriasis. *Eur Acad Dermatol Venereol*. 2017. 7: 1161-1167.
- Rosengarten M, Hadad N, Solomonov Y, Lamprecht S, Levy R. Cytosolic phospholipase A₂ has a crucial role in the pathogenesis of DSS-induced colitis in mice. *Eur J Immunol*. 2016. 46: 400-408.
- Sapirstein A, Bonventre JV. Specific physiological roles of cytosolic phospholipase A₂ as defined by gene knockouts. *Biochim Biophys Acta*. 2000. 1488: 139-148.
- Smith JA, Kauffman CA. Endemic fungal infections in patients receiving tumour necrosis factor- α inhibitor therapy. *Drugs*. 2009. 69: 1403-1415.
- Su B, Karin M. Mitogen-activated protein kinase cascades and regulation of gene expression. *Curr Opin Immunol*. 1996. 8: 402-411.
- Sun H, Charles CH, Lau LF, Tonks NK. MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase *in vivo*. *Cell*. 1993. 75: 487-493.
- Taylor P, Williams R, Feldmann M. Tumour necrosis factor as a therapeutic target for immune-mediated inflammatory diseases. *Curr Opin Biotechnol*. 2004. 15: 557-563.
- Wang J, Yin DP, Liu YX, Baer R, Yin Y. Dual specificity phosphatase 1/CL100 is a direct transcriptional target of E2F-1 in the apoptotic response to oxidative stress. *Cancer Res*. 2007. 67: 6737-6744.
- Wang Z, Xu J, Zhou JY, Liu Y, Wu GS. Mitogen-activated protein kinase phosphatase-1 is required for cisplatin resistance. *Cancer Res*. 2006. 66: 8870-8877.
- Wischmeyer PE, Lynch J, Liedel J, et al. Glutamine administration reduces Gram-negative bacteremia in severely burned patients: a prospective, randomized, double-blind trial versus isonitrogenous control. *Crit Care Med*. 2001. 29: 2075-2080.
- Yang D, Ji HF, Zhang XM, et al. Protective effect of cytosolic phospholipase A₂ inhibition against inflammation and degeneration by promoting regulatory T cells in rats with experimental autoimmune encephalomyelitis. *Mediators Inflamm*. 2014. 2014: 890139-890146.
- Zhao Q, Wang X, Nelin LD, et al. MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxic shock. *J Exp Med*. 2006. 203: 131-140.

<https://doi.org/10.15616/BSL.2021.27.4.223>

Cite this article as: Yoon SY, Jeong SY, Im SY. Glutamine Inhibits TNF- α -induced Cytosolic Phospholipase A₂ Activation via Upregulation of MAPK Phosphatase-1. *Biomedical Science Letters*. 2021. 27: 223-230.