Biochemical and Biophysical Research Communications 437 (2013) 392–396

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Differential regulation of CC chemokine ligand 2 and CXCL8 by antifungal agent nystatin in macrophages

Do-Hyung Kim [a], Byung-Yong Rhim [a], Seong-Kug Eo [b], Koanhoi Kim [a,*]

[a] Department of Pharmacology, School of Medicine, Pusan National University, Yangsan 626-870, Republic of Korea
[b] Laboratory of Microbiology, College of Veterinary Medicine and Bio-Safety Research Institute, Chonbuk National University, Jeonju 561-756, Republic of Korea

ARTICLE INFO

Article history:
Received 21 June 2013
Available online 2 July 2013

Keywords:
CCL2
CXCL8
Nystatin
Macrophages

ABSTRACT

The polyene antifungal antibiotic nystatin can interact with cholesterol, thereby altering the composition of the plasma membrane in eukaryotic cells. We investigated whether nystatin influences responses to the infection by inducing expression of chemokines. THP-1 macrophages rarely expressed CC chemokine ligand 2 (CCL2) and CXCL8. However, nystatin dose-dependently increased CCL2 and CXCL8 expression at the mRNA and protein levels. To understand the molecular mechanisms of the antifungal agent, we identified cellular factors activated by nystatin and those involved in nystatin-induced upregulation of CCL2 and CXCL8. Treatment with nystatin resulted in enhanced phosphorylation of Akt, ERK1/2, p38 MAPK, and JNK. Treatment with cholesterol, LY294002, Akt inhibitor IV, U0126, and SP6001250 resulted in abrogation or significant attenuation of nystatin-induced CCL2 expression. Nystatin-mediated CXCL8 expression was attenuated in the presence of Akt inhibitor IV and SP6001250. These results indicate that exposure of human macrophages to nystatin can lead to differential regulation of CCL2 and CXCL8 via the activation of multiple cellular kinases. We propose that upregulation of CCL2 and CXCL8 contributes to pharmacological effects of nystatin.

1. Introduction

Nystatin is a polyene antifungal agent used treating infections of the skin, mouth, and vigina. It forms barrel-like membrane-spanning pores through its interaction with ergosterol in the membrane of sensitive organisms. Leakage of monovalent ions such as potassium through the pores leads to death of the fungus [1]. Nystatin can also competitively bind to cholesterol in the plasma membrane of eukaryotic cells with relatively low affinity compared to that for ergosterol [2,3]. Such a binding results in sequestration of cholesterol, thereby reducing the ability of cholesterol to interact with other membrane components and altering plasma membrane micro-organization (lipid rafts) [2,4]. Lipid rafts are involved in cellular signaling and function of receptors associated with inflammatory responses [5,6]. Therefore, cholesterol sequestration with nystatin likely affects inflammatory responses in eukaryotic cells. However, whether nystatin influences inflammatory responses has not been extensively examined.

Inflammation is a protective response to remove infectious microorganisms or injurious stimuli and to initiate healing [7]. Critical cellular components involved in initiation and maintenance of the inflammatory response include leukocytes, which typically reside in the blood and migrate to sites of injury or infection [7,8]. Chemokines, which are divided into four families (CC, CXC, CX3C, XC) based on the position of the first 2 cysteine residues, direct leukocyte migration [9]. CC chemokine ligand 2 (CCL2) and CXCL8 (IL-8) are produced during infection or injury and direct the migration of different types of leukocytes. CXCL8 induces neutrophil migration toward the infection site [10], while CCL2 recruits monocytes, memory T cells, and dendritic cells, but not neutrophils [11]. Additionally, CXCL8 and CCL2 are involved in wound healing [12]. Therefore, understanding the mechanisms involved in upregulation of CCL2 and CXCL8 are important since they are key players in inflammatory responses.

In the current study, we investigated whether treatment of eukaryotic cells with the antifungal agent nystatin leads to upregulation of inflammatory chemokines. We found that nystatin induced expression of CCL2 and CXCL8 by THP-1 macrophages. Additionally, we identified cellular molecules showing enhanced activities after treatment with nystatin; we determined their roles in nystatin-mediated expression of CCL2 and CXCL8.

Abbreviations: Akti IV, Akt inhibitor IV; CCL2, CC chemokine ligand 2; ERK1/2, extracellular signal-regulated kinase 1 and 2; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase.

* Corresponding author. Address: Department of Pharmacology, Pusan National University School of Medicine, Busandaehak-ro 49, Mulgeum, Yangsan, Gyeongnam 626-870, Republic of Korea. Fax: +82 51 510 8068.
E-mail address: koanhoi@pusan.ac.kr (K. Kim).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.087

2. Materials and methods

2.1. Cells

THP-1 cells were cultured in RPMI medium 1640 supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 in the presence of penicillin (50 U/mL) and streptomycin (50 μg/mL). THP-1 cells were passaged every 2–3 days to maintain between 1000 and 1,000,000 cells/mL in the culture medium. THP-1 cells between passages 7 and 10 were used for experiments.

2.2. Reagents

Nystatin, LY294002, and SP600125 were purchased from Sigma–Aldrich (St. Louis, MO, USA). U0126, SB202190, and Akt inhibitor IV (Akti IV) were purchased from Cell Signaling Technology (Danvers, MA, USA). Lipopolysaccharide (LPS) and polymyxin B were purchased from InvivoGen (San Diego, CA, USA). The antibody against phosphorylated forms of extracellular signal-regulated kinase 1 and 2 (ERK1/2) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). An antibody against phosphorylated forms of p38 mitogen-activated protein kinase (MAPK) was purchased from R&D Systems (Minneapolis, MN, USA). Antibodies against phosphorylated forms of Akt and phosphorylated forms of c-Jun N-terminal kinase (JNK) were purchased from Cell Signaling Technology.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Commercially available ELISA kits were used according to the manufacturer's instructions (R&D Systems) to determine the amount of CCL2 and CXCL8 released from THP-1 cells. Briefly, recombinant standards of CCL2 and CXCL8 proteins provided in the kit and isolated culture media were added to a plate pre-coated with a monoclonal antibody against each chemokine. After incubation for 2 h, the plate was washed and incubated with an enzyme-linked polyclonal antibody specific for CCL2 or CXCL8. After several washes, the substrate solution was added, and the color intensity was measured. The amount of CCL2 and CXCL8 present in the samples was determined based on a standard curve. Data are expressed as the average ± standard deviation (SD) of triplicate experiments.

2.4. Reverse transcription (RT) and PCR analyses

Total RNAs were reverse-transcribed for 1 h at 42 °C with Moloney Murine Leukemia Virus reverse transcriptase, and non-quantitative and quantitative PCR analyses were performed as previously reported [13]. Primers for CCL2 were 5′-TCTGTGCCTGCTGCTCATAG-3′ (forward) and 5′-CAGATCTCCTTGGCCACAAT-3′ (reverse), and primers for CXCL8 were 5′-TCTGCAGCTCTGTGTGAAGG-3′ (forward) and 5′-AATTTCTGTGTTGGCGCAGT-3′ (reverse). Transcripts of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were amplified as an internal control. Primers for GAPDH were 5-GAGTCAACGGATTTGGTCCT-3 (forward) and 5-TGTGGTCATGAGTCCTTCCA-3 (reverse). The intensities of the CCL2 and CXCL8 were normalized relative to that of GAPDH, which was not altered by any of the treatments. Error bars represent the SD of triplicate experiments.

2.5. Western blot analysis

THP-1 cells were lysed with lysis buffer (1% SDS, 1 mM $NaVO_3$, 10 mM Tris–HCl, pH 7.4) containing protease inhibitors, and supernatants were isolated after centrifugation. Cell lysates containing an equal amount of protein were separated by 12% SDS–PAGE and transferred to polyvinylidene fluoride membranes. After blocking for 1 h in 5% skim milk in 0.1% Tween 20/Tris buffered saline (TBS), membranes were incubated at 4 °C with the appropriate secondary antibody diluted in blocking solution overnight. After washing three times with 0.1% Tween 20/TBS for 10 min each, membranes were incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted in blocking solution. After washing three times with washing buffer for 10 min each, bands were detected using chemiluminescent reagents.

2.6. Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Turkey's multiple comparison tests, using GraphPad PRISM (version 5.0).

3. Results

3.1. Upregulation of CCL2 and CXCL8 in the presence of nystatin

To investigate whether nystatin affects expression of CCL2 and CXCL8 chemokines in macrophages, we performed concentration experiments using THP-1 cells. Results of ELISA revealed that THP-1 cells secreted low amounts of CCL2 and CXCL8 proteins and their secretion remarkably increased in a dose-dependent manner in the presence of nystatin (Fig. 1A). The amount of CCL2 released by THP-1 cells increased from 9.7 pg/mL to 10.2, 63.7, and 1056.9 pg/mL in the presence of 0.1, 1, and 10 μg/mL of nystatin, respectively. Similarly, the amount of CXCL8 secreted increased from 6.1 pg/mL to 29.4, 182.5, and 413.1 pg/mL in the presence of 0.1, 1, and 10 μg/mL of nystatin, respectively. Using RT-PCR and realtime PCR, we evaluated whether nystatin influenced expression of CCL2 and CXCL8 chemokines at the mRNA level (Fig. 1B). Transcription of CCL2 and CXCL8 was weakly detected from THP-1 cells in the absence of nystatin and in the presence of 0.1 μg/mL nystatin. However, elevated levels of transcripts of CCL2 and CXCL8 were observed in the presence of high concentrations of nystatin. Transcription of CCL2 and CXCL8 was induced in the presence of 1 μg/mL nystatin, which was further enhanced in the presence of 10 μg/mL nystatin. We also performed time course experiments. Nystatin-mediated secretion of CCL2 and CXCL8 proteins reached a maximum at 24 h post-treatment and slightly decreased thereafter (Fig. 1C). Transcription of CCL2 and CXCL8 was observed as early as 12 h post-treatment, and the induction persisted to 48 h post-treatment with nystatin (data not shown).

3.2. Upregulation of CCL2 in a cholesterol-dependent fashion

We investigated whether nystatin-induced expression of CCL2 and CXCL8 was influenced in the presence of cholesterol. Treatment with nystatin significantly enhanced secretion of CCL2 and CXCL8 from THP-1 cells, as observed above, and addition of cholesterol resulted in a significant decrease in the secretion of CCL2. The amount of secreted CCL2 showed an increase from 35.3 pg/mL to 1047.3 pg/mL in response to nystatin, which was significantly lowered to 613.8 pg/mL in the presence of 25 μg/mL cholesterol. However, nystatin-mediated CXCL8 secretion was not changed in the presence of cholesterol (Fig. 2A). Cholesterol also attenuated transcription of CCL2 in an identical manner to that observed in ELISA results. Cholesterol attenuated

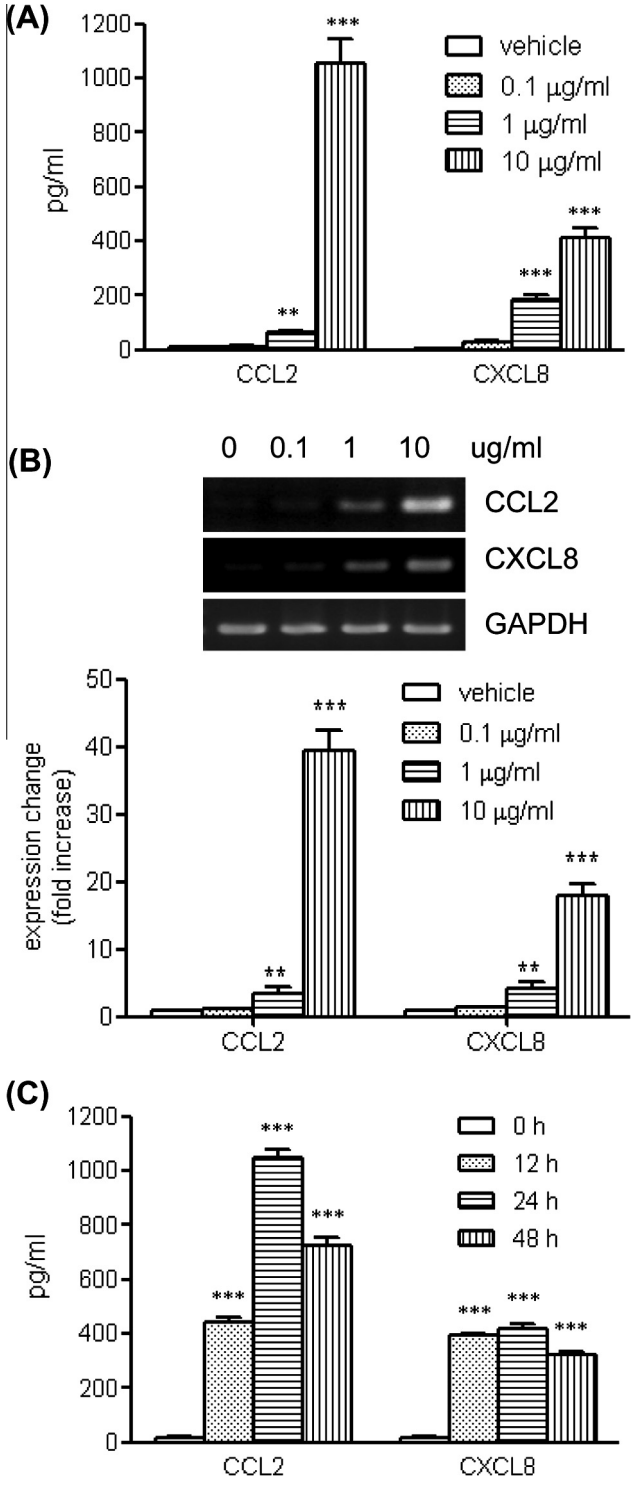

Fig. 1. Enhanced secretion and transcription of CCL2 and CXCL8 by macrophages in response to nystatin. (A, B) THP-1 cells (1×10^6 cells/mL) were incubated for 24 h in the absence or presence of the indicated concentrations of nystatin. (A) The amount of CCL2 and CXCL8 proteins released into the medium was measured by ELISA. $^{**}P < 0.01$ vs. vehicle, $^{***}P < 0.001$ vs. vehicle. (B) Transcripts of CCL2 and CXCL8 genes were amplified by RT-PCR and realtime PCR. $^{**}P < 0.01$ vs. vehicle, $^{***}P < 0.001$ vs. vehicle. (C) THP-1 cells were incubated for the indicated periods in the absence or presence of nystatin (10 μg/mL). The amount of CCL2 and CXCL8 proteins released into the medium was measured by ELISA. $^{***}P < 0.001$ vs. 0 h.

transcription of CCL2 at a high concentration, but nystatin-induced transcription of CXCL8 was not affected by co-treatment with additional cholesterol (Fig. 2B).

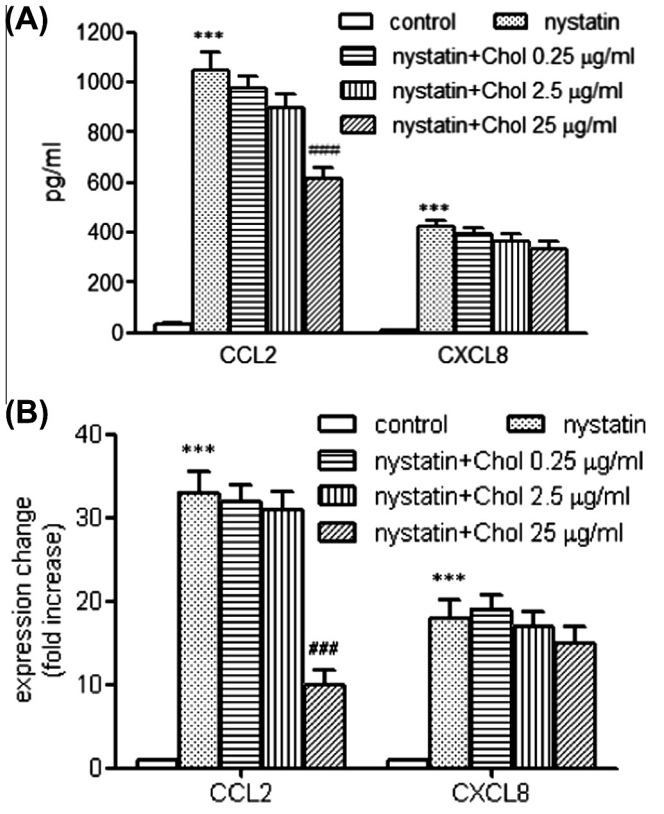

Fig. 2. Effects of cholesterol on secretion and transcription of CCL2 and CXCL8. (A, B) THP-1 cells were stimulated for 24 h with or without nystatin (10 μg/mL) after treatment for 1 h with the indicated amount of cholesterol (Chol). (A) The amount of CCL2 and CXCL8 proteins released into the medium was measured by ELISA. $^{***}P < 0.001$ vs. control. $^{\#\#\#}P < 0.01$ vs. nystatin. (B) Transcripts of the CCL2 and CXCL8 genes were amplified by realtime PCR. $^{***}P < 0.001$ vs. control. $^{\#\#\#}P < 0.01$ vs. nystatin.

3.3. Roles of phosphoinositide 3-kinase (PI3K)/Akt pathway in nystatin-induced expression of CCL2 and CXCL8

Using Western blot analysis, we examined the phosphorylation of Akt to assess whether nystatin affects Akt activity (Fig. 3A). Treatment with nystatin resulted in enhanced phosphorylation of Akt. Increased Akt phosphorylation was observed 40 min post-treatment, with maximal phosphorylation of Akt occurring 60 min post-treatment with nystatin. Akt phosphorylation was sustained for upto 120 min post-treatment with nystatin. We investigated the roles of Akt in expression of CCL2 and CXCL8 using Akti IV, which inhibits Akt activation, and LY294002, which inhibits PI3K, an activator of Akt (Fig. 3B and C). Secretion of CCL2 was significantly reduced in the presence of LY294002 and Akti IV whereas CXCL8 secretion was attenuated in the presence of Akti IV alone. Both inhibitors influenced transcription of the CCL2 and CXCL8 genes in a similar pattern. Transcription of the CCL2 gene induced by treatment with nystatin was significantly attenuated and obligated by treatment with LY294002 and Akti IV, respectively. Transcription of CXCL8 was attenuated by treatment with Akti IV, but not by LY294002.

3.4. Differential roles of MAPKs in nystatin-induced expression of CCL2 and CXCL8

Prior to investigating the roles of MAPKs in nystatin-induced expression CCL2 and CXCL8, we assessed whether nystatin induced activation of MAPKs by detecting phosphorylated forms of ERK1/2, p38 MAPK, and JNK on Western blots (Fig. 4A). Treatment with

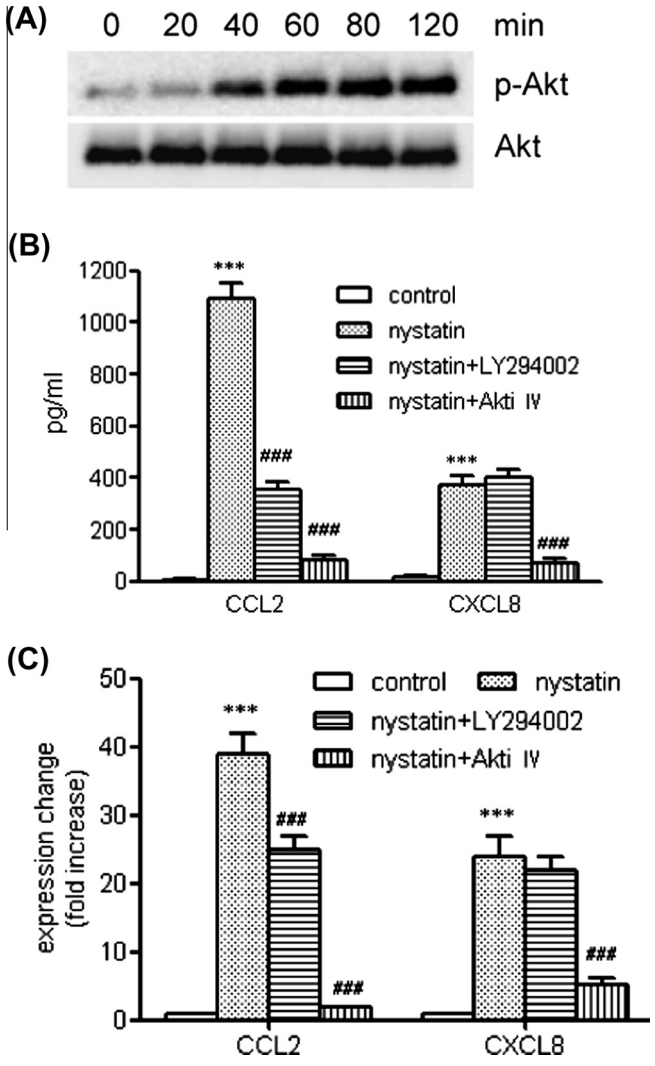

Fig. 3. Effects of inhibitors of PI3K and Akt on nystatin-induced secretion and transcription of CCL2 and CXCL8. (A) THP-1 cells were exposed to nystatin for the indicated periods, after which an equal amount of protein was analyzed by Western blotting using antibodies against phosphorylated and unphosphorylated forms of Akt. THP-1 cells were stimulated for 24 h with or without nystatin (10 μg/mL) after pretreatment for 1 h with LY294002, and Akti IV (10 μM each). (B) The amount of CCL2 and CXCL8 proteins released into the medium was measured by ELISA. $^{***}P < 0.001$ vs. control. $^{\#\#}P < 0.01$ vs. nystatin, $^{\#\#\#}P < 0.001$ vs. nystatin. (C) Transcripts of CCL2 and CXCL8 genes were amplified by realtime-PCR. $^{***}P < 0.001$ vs. control. $^{\#\#\#}P < 0.001$ vs. nystatin.

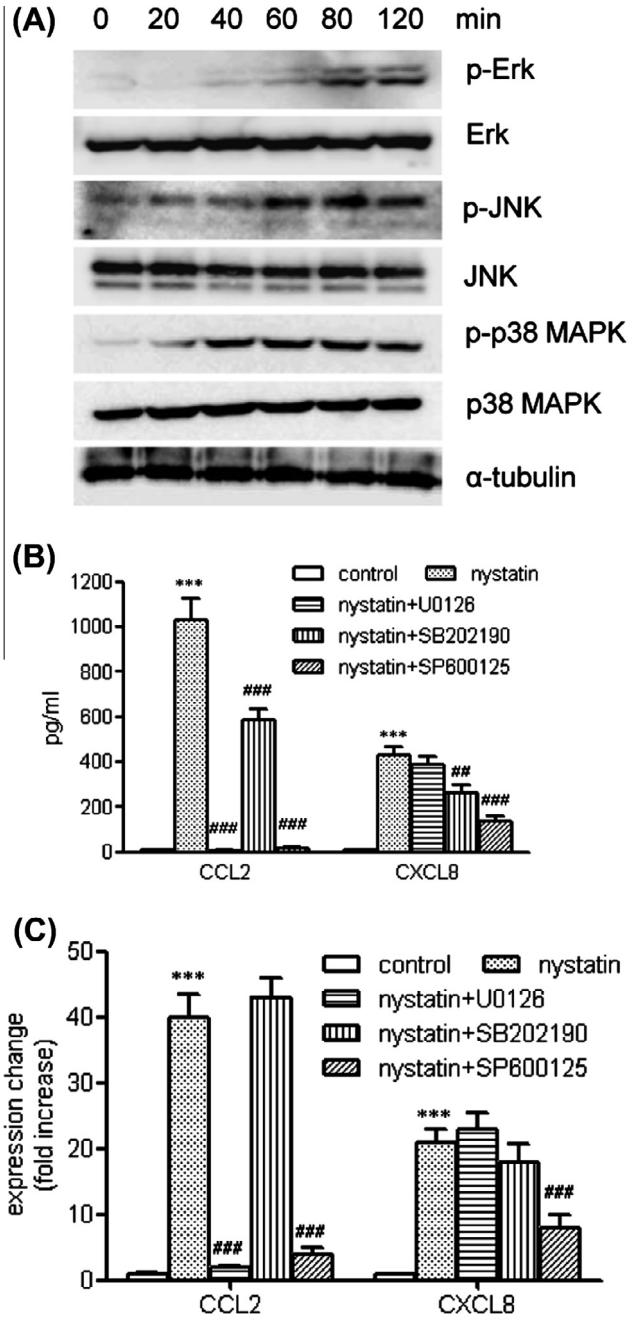

Fig. 4. Effects of inhibitors of MAPKs on nystatin-induced secretion and transcription of CCL2 and CXCL8. (A) THP-1 cells were exposed to nystatin for the indicated periods, after which an equal amount of protein was analyzed by Western blotting using antibodies against phosphorylated and unphosphorylated forms of ERK1/2, p38 MAPK, and JNK. THP-1 cells were stimulated for 24 h with or without nystatin (10 μg/mL) after pretreatment for 1 h with the indicated MAPKs inhibitors (10 μM each). (B) The amount of CCL2 and CXCL8 proteins released into the medium was measured by ELISA. $^{***}P < 0.001$ vs. control. $^{\#\#}P < 0.01$ vs. nystatin, $^{\#\#\#}P < 0.001$ vs. nystatin. (C) Transcripts of CCL2 and CXCL8 genes were amplified by realtime-PCR. $^{***}P < 0.001$ vs. control. $^{\#\#\#}P < 0.001$ vs. nystatin.

nystatin resulted in enhanced phosphorylation of the three kinases. Increased phosphorylation of ERK1/2 and JNK was observed 60 min post-treatment with nystatin, which was sustained for upto 120 min post-treatment. Increased phosphorylation of p38 MAPK occurred 40 min post-treatment with nystatin, which reached a maximum level at 80 min post-treatment and decreased thereafter.

We investigated whether MAPKs play roles in upregulating CCL2 and CXCL8 by using inhibitors of SB202190 (a p38 MAPK inhibitor), SP600125 (a JNK inhibitor), and U0126 (an ERK pathway inhibitor) (Fig. 4B and C). Nystatin-mediated secretion of CCL2 was obligated by treatment with U0126 and SP600125 and significantly reduced in the presence of SB202190. Nystatin-mediated secretion of CXCL8 was significantly attenuated by treatment with SB202190 and SP600125, but not by U0126. We also investigated the effects of inhibitors on the transcription of CCL2 and CXCL8. Nystatin-induced transcription of CCL2 was inhibited by treatment with U0126 and SP600125, while transcription of CXCL8 was attenuated by SP600125 alone.

4. Discussion

In this study, we demonstrated that treatment of THP-1 human macrophages with nystatin resulted in upregulation of CCL2 and CXCL8 chemokines at both the mRNA and protein levels. Because nystatin is able to sequester cholesterol on plasma membranes [2,4], we examined whether the presence of additional cholesterol could modify the effects of nystatin on expression of chemokines. Addition of cholesterol led to attenuation of CCL2 expression,

whereas nystatin-induced expression of CXCL8 remained unchanged, indicating that, compared with CXCL8, nystatin-induced CCL2 expression was likely to be affected by cholesterol. These results suggest that nystatin can induce cholesterol-dependent and -independent expression of chemokines.

A small amount of endotoxin is sufficient to induce chemokine expression including CCL2 and CXCL8 in THP-1 monocytic cells [14]. Therefore, we investigated the question of whether contaminating endotoxin, if any, contributed to expression of CCL2 and CXCL8 in our experiments. To rule out that possibility, we used polymyxin B which prevents biological effects mediated by LPS by binding to it [15]. We found that treatment with polymyxin B resulted in blockage of CCL2 and CXCL8 expression induced by LPS (Supplemental data). Polymyxin B, however, did not attenuate expression of the chemokines induced by nystatin. These results indicate that endotoxin did not play a role in chemokine expression induced by nystatin.

The PI3K/Akt pathway not only has a fundamental effect on cholesterol homeostasis [16], but can also regulate acute and chronic inflammatory processes [17]. Therefore, we attempted to determine whether the PI3K/Akt pathway played roles in nystatin-induced expression of CCL2 and CXCL8. Upon exposure to nystatin, phosphorylation of Akt was enhanced. These results indicate that treatment with nystatin activates the PI3K/Akt pathway as PI3K activation leads to phosphorylation/activation of Akt kinase [18]. To assess the roles of PI3K/Akt, we used two pharmacological inhibitors-LY294002 and Akti IV. LY294002, a morpholine derivative of quercetin, potently inhibits PI3Ks [19]. Akti IV inhibits Akt activation by targeting the ATP binding site of a kinase upstream of Akt, but downstream of PI3K [20]. Nystatin-induced CCL2 expression was significantly attenuated at the transcriptional and protein levels by both inhibitors, whereas CXCL8 expression was attenuated by Akti IV, but not by LY294002. Therefore, Akt appears to play critical roles in nystatin-induced expression of CCL2 and CXCL8. Additionally, these results suggest that nystatin induces expression of chemokines by activating Akt via both PI3K-dependent and -independent pathways.

MAPKs are serine/threonine-specific protein kinases that mediate inflammation by inducing chemokine production in response to stimuli, including growth factors and oxidative stress [21], and are activated via PI3K/Akt when cholesterol homeostasis is disrupted [22]. Therefore, we examined activation of the kinases and their involvement in upregulating CCL2 and CXCL8 in response to nystatin. Treatment with nystatin resulted in elevated phosphorylation of ERK1/2, p38 MAPK, and JNK, indicating activation of the three MAPKs. Expression of CCL2 was attenuated by inhibition of the ERK pathway or JNK, while CXCL8 expression was attenuated by inhibition of JNK at the mRNA and protein levels. Additionally, inhibition of p38 resulted in attenuated secretion of chemokines, without affecting transcription, indicating active involvement of p38 MAPKs in chemokine secretion. These data suggest that each MAPK has distinct role in expression of CCL2 and CXCL8 and activation of multiple MAPKs is required for maximal release of CCL2 and CXCL8 in response to nystatin.

We demonstrated that the antifungal agent nystatin activated multiple cellular signaling molecules, including Akt, ERK1/2, p38 MAPK, and JNK in human macrophages. Moreover, nystatin induced upregulation of CCL2 and CXCL8 by macrophages, and inhibition of the kinase resulted in differential upregulation of CCL2 and CXCL8. Because MAPK pathways are interconnected with PI3K/Akt [23], these molecules may act in an independent or in a cooperative manner. We did not examine the manner by which these kinases function. To further understand the molecular mechanisms through which nystatin induces expression of CCL2 and CXCL8, the types of connections or crosstalks among kinases should be examined.

Acknowledgments

This work was supported by the Bio-Scientific Research Grant funded by the Pusan National University (PNU, Bio-Scientific Research Grant) (PNU-2010-101-256).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.087.

References

[1] G. Fujii, J.E. Chang, T. Coley, B. Steere, The formation of amphotericin B ion channels in lipid bilayers, Biochemistry 36 (1997) 4959–4968.
[2] J. Bolard, How do the polyene macrolide antibiotics affect the cellular membrane properties?, Biochim Biophys. Acta 864 (1986) 257–304.
[3] L. Silva, A. Coutinho, A. Fedorov, M. Prieto, Competitive binding of cholesterol and ergosterol to the polyene antibiotic nystatin. A fluorescence study, Biophys. J. 90 (2006) 3625–3631.
[4] H. Park, Y.M. Go, P.L. St John, M.C. Maland, M.P. Lisanti, D.R. Abrahamson, H. Jo, Plasma membrane cholesterol is a key molecule in shear stress-dependent activation of extracellular signal-regulated kinase, J. Biol. Chem. 273 (1998) 32304–32311.
[5] M.B. Fessler, J.S. Parks, Intracellular lipid flux and membrane microdomains as organizing principles in inflammatory cell signaling, J. Immunol. 187 (2011) 1529–1535.
[6] X. Zhu, J.S. Owen, M.D. Wilson, H. Li, G.L. Griffiths, M.J. Thomas, E.M. Hiltbold, M.B. Fessler, J.S. Parks, Macrophage ABCA1 reduces MyD88-dependent toll-like receptor trafficking to lipid rafts by reduction of lipid raft cholesterol, J. Lipid Res. 51 (2010) 3196–3206.
[7] J. Hart, Inflammation. 1: Its role in the healing of acute wounds, J. Wound Care 11 (2002) 205–209.
[8] C.H. Wong, B. Heit, P. Kubes, Molecular regulators of leucocyte chemotaxis during inflammation, Cardiovasc. Res. 86 (2010) 183–191.
[9] S.J. Allen, S.E. Crown, T.M. Handel, Chemokine: receptor structure, interactions, and antagonism, Annu. Rev. Immunol. 25 (2007) 787–820.
[10] N. Li Jeon, H. Baskaran, S.K. Dertinger, G.M. Whitesides, L. Van de Water, M. Toner, Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device, Nat. Biotechnol. 20 (2002) 826–830.
[11] S.L. Deshmane, S. Kremlev, S. Amini, B.E. Sawaya, Monocyte chemoattractant protein-1 (MCP-1): an overview, J. Interferon Cytokine Res. 29 (2009) 313–326.
[12] R. Gillitzer, M. Goebeler, Chemokines in cutaneous wound healing, J. Leukoc. Biol. 69 (2001) 513–521.
[13] S.H. Kang, J.H. Lee, K.H. Choi, B.Y. Rhim, K. Kim, Roles of ERK and NF-kappaB in Interleukin-8 expression in response to heat shock protein 22 in vascular smooth muscle cells, Korean J. Physiol. Pharmacol. 12 (2008) 171–176.
[14] L.M. Harrison, C. van den Hoogen, W.C. van Haaften, V.L. Tesh, Chemokine expression in the monocytic cell line THP-1 in response to purified shiga toxin 1 and/or lipopolysaccharides, Infect. Immun. 73 (2005) 403–412.
[15] L.S. Cardoso, M.I. Araujo, A.M. Goes, L.G. Pacifico, R.R. Oliveira, S.C. Oliveira, Polymyxin B as inhibitor of LPS contamination of *Schistosoma mansoni* recombinant proteins in human cytokine analysis, Microb. Cell Fact. 6 (2007) 1.
[16] X. Du, I. Kristiana, J. Wong, A.J. Brown, Involvement of Akt in ER-to-Golgi transport of SCAP/SREBP: a link between a key cell proliferative pathway and membrane synthesis, Mol. Biol. Cell 17 (2006) 2735–2745.
[17] C. Rommel, M. Camps, H. Ji, PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond?, Nat Rev. Immunol. 7 (2007) 191–201.
[18] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer, Nat. Rev. Cancer 2 (2002) 489–501.
[19] C.J. Vlahos, W.F. Matter, K.Y. Hui, R.F. Brown, A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), J. Biol. Chem. 269 (1994) 5241–5248.
[20] T.R. Kau, F. Schroeder, S. Ramaswamy, C.L. Wojciechowski, J.J. Zhao, T.M. Roberts, J. Clardy, W.R. Sellers, P.A. Silver, A chemical genetic screen identifies inhibitors of regulated nuclear export of a forkhead transcription factor in PTEN-deficient tumor cells, Cancer Cell 4 (2003) 463–476.
[21] B. Kaminska, MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits, Biochim. Biophys. Acta 1754 (2005) 253–262.
[22] X. Chen, M.D. Resh, Activation of mitogen-activated protein kinase by membrane-targeted Raf chimeras is independent of raft localization, J. Biol. Chem. 276 (2001) 34617–34623.
[23] E. Aksamitiene, A. Kiyatkin, B.N. Kholodenko, Cross-talk between mitogenic Ras/MAPK and survival PI3K/Akt pathways: a fine balance, Biochem. Soc. Trans. 40 (2012) 139–146.