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Review Article

Role of azithromycin in antiviral treatment: enhancement of interferon-dependent antiviral pathways and mitigation of inflammation may rely on inhibition of the MAPK cascade?

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Abstract: Azithromycin is a macrolide-type antibiotic used against a broad range of bacterial infection, such as respiratory tract, skin, ear, eye infections, and sexually transmitted diseases. The ongoing severe acute respiratory syndrome (SARS) mediated by Corona Virus 2 (CoVid19) is a global health concern and various countries witnessed the loss of precious human life. In fall 2020, the absence of specific suitable medication or vaccine is still a major cause of concern to fight the pandemic while different countries have already started using their own medication and available resources to save the life of their citizens. At the present, in many countries around the world, we witnessed the use of the antibiotic azithromycin towards the medication of CoVid19; even its effect on anti CoVid19 is still controversial. This mini review aims to address whether azithromycin can affect molecular pathway involved in inflammatory immunity upon viral infection, to find out the rationale behind the use of azithromycin in the treatment of CoVid19. Overall, the data show that the mechanism of action of azithromycin in viral infection may be dependent on a global amplification of the interferon-dependent pathways mediating antiviral responses, leading to a reduction of viral replication, together with a strong impairment of the inflammatory pathways, relying on MAPK cascade inactivation.

Keywords: SARS, CoVid19, azithromycin, interferon, inflammation, mitogen activated protein kinase

Introduction

Coronavirus Disease 2019 (COVID-19), a respiratory illness caused by a novel β-coronavirus called SARS-CoV-21, was first identified in the Wuhan City, Hubei Province of China in December 2019, subsequently World Health Organization (WHO) declared COVID-19 as a pandemic on March 11, 2020 (https://www.medscape.com/viewarticle/924596). Thereafter the disease has spread across the globe with its unique characteristic of fast spreading and causing more death than previous known coronavirus. Till date this pandemic has affecting nearly 40 million of cases, causing the death of more than 1,000,000 people in 215 countries with no effective treatments and only partial understanding of the immunological response to the virus. Nevertheless, some countries have begun to use the macrolide antibiotic azithromycine, alone and in combination with other medications for treatment of COVID-19 patients (Source: ICMR, Indian Council of Medical Research). Indeed, azithromycin exhibits anti-viral activity aside from its antibacterial effect, providing additional therapeutic benefit. Considering the pharmacokinetics of azithromycin, it achieves high tissue concentration and hence able to deliver the compound to the site of viral infection, especially the lungs [1]. It is more stable in acidic media, with a single day dosage due to its long half-life period (11-14 hrs

for single dosage and 68 hrs for multiple dosages). The metabolism of azithromycin is very slow and does not inhibit the cytochrome p450 enzyme system [2, 3]. Azithromycin inhibits the protein synthesis by binding to the 50S subunit of the bacterial ribosome, where its prevents the transfer of tRNA from A to P site of the ribosome and aborts the growth of polypeptide [1].

Azithromycin efficacy in the treatment of COVID-19 remains unclear. Very recently, the Brasilian COALITION II clinical trial showed that adding azithromycin to standard of care treatment (which included hydroxychloroquine) did not improve clinical outcomes [4]. These data suggest that azithromycin might not be beneficial in hospitalised patients with aggressive disease. On the other hand, a French study showed that azithromycin reinforces the significant reduction/disappearance of viral load in COVID-19 patients treated with hydroxychloroquine [5]. Therefore, the role of azithromycin in COVID-19 should be strengthened by additional placebo-controlled trials in hospitalised patients, earlier in the disease progress. Because of its large use in the therapy for COVID-19, establishing whether azithromycin is helpful in earlier stages of the disease is an important research priority [6].

Since it is counterintuitive to use an antibiotic to treat viral infection, more information on the molecular mechanisms exerted by azithromycin is needed before any conclusions can be made regarding the possible benefits and risks of using azithromycin in patients with COVID-19 or with other viral pandemic events. This short Review aims to highlight recent data in the literature showing that azithromycin can affect inflammatory immunity, exploiting molecular pathways involved in immunomodulation during viral infection, to fill a current research gaps, with the potential future developments in the field. Indeed, azithromycin, among the molecular mechanisms involved in viral infection, can cause a significant increase in interferon (IFN)-mediated antiviral responses leading to a reduction of viral replication, together with a strong impairment of the inflammatory pathways, both of them relying on a tight control of MAPK cascade activation.

Azithromycin action against viral infection

Azithromycin could act on different points of the viral cycle, exhibiting both antiviral and immunomodulatory properties. For example, it can inhibit SARS-CoV-2 binding to the human ACE2 receptor, by associating to the spike protein of SARS-CoV-2. Unexpectedly, azithromycin is similar to the sugar moiety of GM1, a lipid raft ganglioside acting as a host attachment cofactor for respiratory viruses. Therefore, it may interacts with the ganglioside-binding domain of SARS-CoV-2 spike protein, acting as a competitive inhibitor of SARS-CoV-2 attachment to the host-cell membrane [7] (**Figure 1**). Azithromycin may also affect membrane fusion, endocytosis, and lysosomal protease activation, as cathepsins or furins, implicated in the cleavage of the spike protein of SARS-CoV-2 (**Figure 1**). On the other hand it can stabilize epithelial tight junctions or decrease mucus hyper secretion, which may improve mucociliary clearance [8].

Regarding the immunomodulatory properties and the involved molecular pathways, azithromycin treatment can impact on T-lymphocyte, suppressing CD4+ T-cell activation by inhibiting m-TOR activity [9]. Moreover, azithromycin down-regulates NK cytotoxicity and cytokine production upon mycoplasma infection [10]. Azithromycin also down-regulates cytokines and chemokines production, and may increase apoptosis by shifting alveolar macrophages to anti-inflammatory phenotype [8]. In terms of affected molecular pathways, in addition to m-TOR, in a murine model of chronic asthma, long-term azithromycin treatment ameliorates not only airway inflammation but also airway remodelling by influencing both the Mitogen Activated Protein Kinase (MAPK) and the NF-κB signal pathways, thus being an effective adjuvant therapy in a chronic, severe asthma with remodelling airway [11] (**Figure 1**).

Azithromycin has proved to possess antiviral effect against a broad variety of viruses, including rhinovirus (RN), influenza, and Zika. Asthma exacerbations caused by RN leads to higher rate of morbidity and mortality. In addition, asthma patient also exhibit low level production of IFN-α and IFN-β that are required to fight viral infection [12, 13]. To overcome the problem, researchers have implemented the use of azithromycin to combine both antibacterial and anti-inflammatory activities [14, 15]. Study led by Gielen et al., (2010) reported that in primary human bronchial epithelial cells, azithromycin significantly increased RN1B- and RN16-

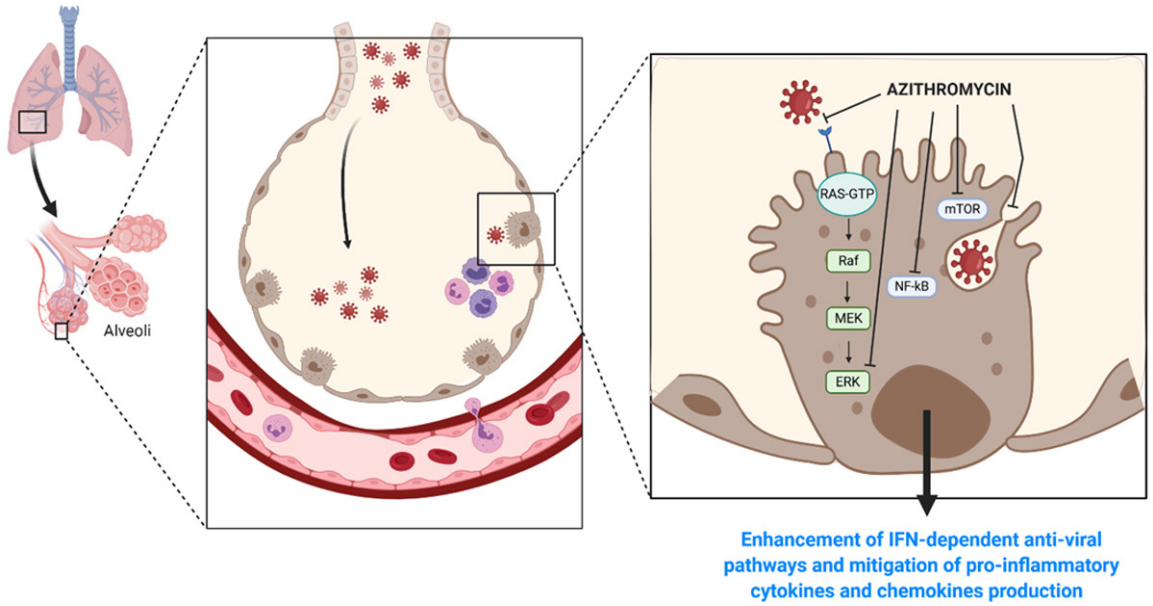

Figure 1. Antiviral mechanisms of azithromycin. In this Figure, a Club cells, also known as bronchiolar exocrine cells, and formerly known as Clara cells, is magnified. These cells are dome-shaped cells with short microvilli, and they are found in the small airways (bronchioles) of the lung. Upon Covid-19 viral infection, Azithromycin can mimic the GM1 ganglioside, acting as a competitive inhibitor of SARS-CoV-2 attachment to the host-cell membrane Human Ace2 receptor. Moreover, azithromycin impairs viral particle endocytosis. In the context of immunomodulatory properties, azithromycin down-regulates the MAPK/ERK pathway, as well NF-κB and m-TOR activation, leading to enhancement of interferon-dependent antiviral pathways and mitigation of inflammation, with decreased production of cytokines and chemokines, such as IL-1β, IL-8, TNF-α, and GM-CSF. (Created with BioRender.com.).

induced IFN and IFN-stimulated gene expression and protein production with concurrent reduction of viral replication and release [15], thus demonstrating that azithromycin has anti-RN activity in bronchial epithelial cells and, during RN infection, increases the production of IFN-stimulated pathways. Moreover, RV infection is also the major cause of chronic obstructive pulmonary disease (COPD) exacerbations and may contribute to the development into severe stages of COPD. By comparing primary bronchial epithelial cells from COPD donors and healthy individuals it has been shown that azithromycin transiently increased RV16-induced expression of IFN-β in COPD cells but not in healthy epithelial cells. Azithromycin also decreased viral load, supporting azithromycin's emerging role in prevention of exacerbations of COPD [16]. Similarly, in cystic fibrosis (CF) patients, the viral infection in the respiratory tract also leads to pulmonary exacerbations and it has been reported that azithromycin-mediated treatment reduces RV replication in CF bronchial epithelial cells, possibly through the amplification of the antiviral response mediated by the IFN pathway [17]. On the other hand, in the absence of acute RN viral infection, long term and low dose of azithromycin (250 mg/day) is associated with the down-regulation of genes associated with antigen presentation, IFN-regulated genes, and inflammatory pathways [18]. In lung allograft recipient patients, azithromycin proved to reduce the inflammation in airways due to RV infection [19]. Prophylactic treatment with azithromycin was also shown to mitigate the detrimental responses as well [19].

Influenza A (H1N1) pdm09 virus was the causal factor of the recent global flu pandemic in 2009, which claimed several hundred lives, and currently it also causes seasonal and annual epidemic as well. Application of anti-influenza drug neuraminidase and Matrix protein 2 (M2) channel inhibitors, caused drug resistance against H1N1. However, in vivo and in vitro study revealed that application of azithromycin inhibits the viral progeny and replication [20]. Administering of a single intranasal azithromycin treatment to mice infected with H1N1 virus, successfully reduced viral load in the lungs and relieved hypothermia, which was induced by

infection. Moreover, in vitro, progeny virus replication was remarkably inhibited by treating viruses with azithromycin before infection, by blocking internalization into host cells during the early phase of infection, and targeting newly budded progeny virus from the host cells, inactivating their endocytic pathway [20].

Another example comes from Zika virus infection, where application of azithromycin reduced the viral proliferation in the glial cell population, the more susceptible to the Zika virus infection [21]. In foetuses and neonates, treatment with azithromycin (50 mg/L) attenuates replication of Zika virus [22].

Azithromycin also showed potential anti-viral and anti-inflammatory activity in a mouse model of viral bronchiolitis, one of the most substantial health burdens for infants and young children worldwide, where it attenuates airways inflammation in the pre-bronchial space, airway lumen, and alveolar space [23], also decreasing the accumulation of inflammatory cells (macrophage, lymphocytes, and neutrophils) in the lung tissue [24]. While such clinical outcomes are promising for the future management of these diseases, however, the underlying mechanisms remain still largely unclear.

Signalling cascade of azithromycin: involvement of the MAPK cascade

The MAPK, including Extracellular Receptor like Kinases (ERK), c-Jun NH_2-terminal Kinase (JNK), and p^{38}MAPK, are important signalling molecule associated with diverse cellular mechanisms in the cell [25].

It has been known from many years that the MAPK cascade is associated with the Interleukin (IL)-8 gene expression, a prototypic human chemokine, as the founding member of the chemokine superfamily. In healthy tissues, IL-8 is barely detectable, but it is rapidly induced by 10 to 100-fold in response to proinflammatory cytokines such as tumour necrosis factor (TNF) or IL-1, bacterial or viral products, and cellular stress [26, 27]. The p^{38}MAPK and ERK increase IL-8 expression in human bronchial cells, while ERK has been reported to activate the NF-κB-p65 pathway in airway bronchial cells [26-28]. The connection between azithromycin and the MAPK cascade originated from the observation that azithromycin can modulate ERK phosphorylation and interleukin-8 (IL-8)/GM-CSF production in human bronchial cells [29].

Cytokines and chemokines play critical roles in the pathogenesis of asthma. Indeed, azithromycin, frequently used in asthmatic children with lower respiratory tract infection, inhibits the accumulation of neutrophils in lung airways by affecting interleukin-17 downstream signal, and by inhibiting the release of neutrophil mobilizing cytokines MIP-2, CXCL-5, and GM-CSF [30]. In THP-1 human monocytic cells, azithromycin suppressed Lipopolysaccharide (LPS)-induced Macrophage-Derived Chemokines (MDC) expression via the JNK and the NF-κB-p65 pathways. Azithromycin also suppressed LPS-induced IFN-inducible protein-10 (IP-10/CXCL10), a T helper (Th)1-related chemokine contributing to asthmatic airway inflammation and hypersensitivity [31] via the MAPK-JNK/ERK and the NFκB-p65 pathway [32], thus benefiting asthmatic patients by suppressing chemokines expression. Moreover, when treated with bacterial super antigen, azithromycin (5 μg/ml) suppress the proliferation, interleukin production and the MAPK pathway in human mononuclear cells [30]. Therefore, the suppression of MAPK pathway by azithromycin [30] might impact on the activation of viral progeny. Indeed, the concentration of TNF-α, and ILs (IL-2, 4, 5, and 10) in Concanavalin A-stimulated PBMC decreased by 65-68% in the presence of azithromycin [30]. Azithromycin was also reported to modulate the ERK-mediated pathways and to inhibit the production of inflammatory cytokines and chemokines in epithelial cells from infertile women with recurrent *Chlamydia trachomatis* infection [33].

It is already known that the MAPK pathways may be altered/involved in viral infection, as a cellular signalling pathways exploited by viruses for their own replication machinery, including translation, transport across nuclear membrane, as well as capsid assembly, and spreading, and reactivation of virus latency [33, 34]. The attenuated MAPK pathway is the rate limiting step for the activation and progeny of viral infection and dampening its activation can directly inactivate the pathway that allow viral progeny [34]. On the basis of these data, the inhibition of MAPK cascade by azithromycin might play an important role towards the Co-

Vid19 infection. Moreover, azithromycin in LPS-stimulated THP-1 cells decreased the TNF-α production, together with a decrease in p38 MAPK activation [35]. However, the level of IKB-α (an inhibitor of the NF-κB pathway) remained unaltered [35]. The fact that the LPS-induced TNF-α release from the THP-1 cells was inhibited by heat shock protein 70 (HSP-70) inhibitors, and that the induction of HSP-70 by LPS get attenuated by azithromycin [35], demonstrate that azithromycin restrains the production of TNF-α by modifying HSP-70 and p38-related signalling pathway [35].

Moreover, in human airway smooth muscle cells, bronchial vascular changes associated with elevated expression of pro-angiogenic fibroblast growth factor FGF-1 and FGF-2 get influenced by the application of azithromycin [36], indicating that azithromycin attenuates FGF-induced VEGF via reducing p38 MAPK signalling [36]. Neonatal inflammation, which is mediated in part by TLR and inflammasome signalling, can contribute to host defence against infection. Azithromycin also exerts immune-modulatory effects on toll like-receptor (TLR)- and/or inflammasome-mediated cytokine production in human new-born and adult whole blood in vitro [37]. Indeed, azithromycin alone, at clinically relevant 2.5-20 μM concentration, or in combination with the phosphodiesterase pentoxifylline, and with the steroid dexamethasone, strongly affects TLR- and/or inflammasome-mediated cytokine production. The authors suggest that its anti-inflammatory mechanisms may rely on modulation of NF-κB, MAPK, TLR, IFNs, and inflammasome pathways, and that dampening of these pathways may contribute to the observed synergistic immune-modulatory effects of these drug combinations.

Conclusions

In an era of precision medicine, the unique anti-inflammatory profiles of azithromycin could pave the way for more balanced anti-inflammatory drug combinations suitable to fight viral infection. During the global crisis such as that due to CoVid19, the need to discover new drugs or to repositioning old medicines for new uses is urgent. Many patients around the world have been already treated with these drugs in this pandemic situation. In fall 2020, still, there is no specific treatment for disease caused by the novel coronavirus. Some treatments have produced different results in different setting, raising the need for larger, more rigorously designed studies to clear up the confusion. Therefore, given the safety profile of azithromycin, its use together with specific MAPK cascade inhibitors may be tested in the viral infection, in particular against SARS-Cov-2 infection. Future studies, including animal models and clinical trials, should assess the safety and efficacy of these combinations in treating CoVid19 patients.

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Disclosure of conflict of interest

None.

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References

[1] Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM and Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. Pharmacol Ther 2014; 143: 225-245.

[2] Amsden GW. Macrolides versus azalides: a drug interaction update. Ann Pharmacother 1995; 29: 906-917.
[3] Amsden GW. Anti-inflammatory effects of macrolides-an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? J Antimicrob Chemother 2005; 55: 10-21.
[4] Furtado RHM, Berwanger O, Fonseca HA, Corrêa TD, Ferraz LR, Lapa MG, Zampieri FG, Veiga VC, Azevedo LCP, Rosa RG, Lopes RD, Avezum A, Manoel ALO, Piza FMT, Martins PA, Lisboa TC, Pereira AJ, Olivato GB, Dantas VCS, Milan EP, Gebara OCE, Amazonas RB, Oliveira MB, Soares RVP, Moia DDF, Piano LPA, Castilho K, Momesso RGRAP, Schettino GPP, Rizzo LV, Neto AS, Machado FR and Cavalcanti AB. Azithromycin in addition to standard of care versus standard of care alone in the treatment of patients admitted to the hospital with severe COVID-19 in Brazil (COALITION II): a randomised clinical trial. Lancet 2020; 396: 959-967.
[5] Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Tissot Dupont H, Honoré S, Colson P, Chabrière E, La Scola B, Rolain JM, Brouqui P and Raoult D. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020; 56: 105949.
[6] Oldenburg CE and Doan T. Azithromycin for severe COVID-19. Lancet 2020; 396: 936-937.
[7] Fantini J, Chahinian H and Yahi N. Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: what molecular dynamics studies of virus-host interactions reveal. Int J Antimicrob Agents 2020; 56: 106020.
[8] Echeverría-Esnal D, Martin-Ontiyuelo C, Navarrete-Rouco ME, De-Antonio Cuscó M, Ferrández O, Horcajada JP and Grau S. Azithromycin in the treatment of COVID-19: a review. Expert Rev Anti Infect Ther 2020; 1-17.
[9] Ratzinger F, Haslacher H, Poeppl W, Hoermann G, Kovarik JJ, Jutz S, Steinberger P, Burgmann H, Pickl WF and Schmetterer KG. Azithromycin suppresses CD4+ T-cell activation by direct modulation of mTOR activity. Sci Rep 2014; 4: 7438.
[10] Lin SJ, Yan DC, Lee WI, Kuo ML, Hsiao HS and Lee PY. Effect of azithromycin on natural killer cell function. Int Immunopharmacol 2012; 13: 8-14.
[11] Kang JY, Jo MR, Kang HH, Kim SK, Kim MS, Kim YH, Kim SC, Kwon SS, Lee SY and Kim JW. Long-term azithromycin ameliorates not only airway inflammation but also remodeling in a murine model of chronic asthma. Pulm Pharmacol Ther 2016; 36: 37-45.
[12] Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PAB, Bartlett NW, Kebadze T, Mallia P, Stanciu LA, Parker HL, Slater L, Lewis-Antes A, Kon OM, Holgate ST, Davies DE, Kotenko SV, Papi A and Johnston SL. Role of deficient type III interferon-λ production in asthma exacerbations. Nat Med 2006; 12: 1023-1026.
[13] Wark PAB, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST and Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med 2005; 201: 937-947.
[14] Chinali G, Nyssen E, Giambattista MD and Cocito C. Inhibition of polypeptide synthesis in cell-free systems by virginiamycin S and erythromycin. Evidence for a common mode of action of type B synergimycins and 14-membered macrolides. Biochim Biophys Acta 1988; 949: 71-78.
[15] Gielen V, Johnston SL and Edwards MR. Azithromycin induces anti-viral responses in bronchial epithelial cells. Eur Respir J 2010; 36: 646-654.
[16] Menzel M, Akbarshahi H, Bjermer L and Uller L. Azithromycin induces anti-viral effects in cultured bronchial epithelial cells from COPD patients. Sci Rep 2016; 6: 28698.
[17] Schögler A, Kopf BS, Edwards MR, Johnston SL, Casaulta C, Kieninger E, Jung A, Moeller A, Geiser T, Regamey N and Alves MP. Novel antiviral properties of azithromycin in cystic fibrosis airway epithelial cells. Eur Respir J 2015; 45: 428-439.
[18] Baines KJ, Wright TK, Gibson PG, Powell H, Hansbro PM and Simpson JL. Azithromycin treatment modifies airway and blood gene expression networks in neutrophilic COPD. ERJ Open Res 2018; 4: 00031-2018.
[19] Ling KM, Hillas J, Lavender MA, Wrobel JP, Musk M, Stick SM and Kicic A. Azithromycin reduces airway inflammation induced by human rhinovirus in lung allograft recipients. Respirology 2019; 24: 1212-1219.
[20] Tran DH, Sugamata R, Hirose T, Suzuki S, Noguchi Y, Sugawara A, Ito F, Yamamoto T, Kawachi S, Akagawa KS, Ōmura S, Sunazuka T, Ito N, Mimaki M and Suzuki K. Azithromycin, a 15-membered macrolide antibiotic, inhibits influenza A (H1N1) pdm09 virus infection by interfering with virus internalization process. J Antibiot 2019; 72: 759-768.
[21] Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT, Sandoval-Espinosa C, Mancia Leon WR, Krencik R, Ullian EM, Spatazza J, Pollen AA,

Mandel-Brehm C, Nowakowski TJ, Kriegstein AR and DeRisi JL. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proc Natl Acad Sci U S A 2016; 113: 14408-14413.

[22] Bosseboeuf E, Aubry M, Nhan T, de Pina JJ, Rolain JM, Raoult D and Musso D. Azithromycin inhibits the replication of Zika virus. Journal of Antivirals & Antiretrovirals 2018; 10: 6-11.

[23] Beigelman A, Mikols CL, Gunsten SP, Cannon CL, Brody SL and Walter MJ. Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis. Respir Res 2010; 11: 90.

[24] Beigelman A, Mikols CL, Gunsten SP, Cannon CL, Brody SL and Walter MJ. Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis. Respir Res 2010; 11: 1-11.

[25] Kim EK and Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta 2010; 1802: 396-405.

[26] Dong C, Davis RJ and Flavell RA. Signaling by the JNK group of MAP kinases. J Clin Immunol 2001; 21: 253-257.

[27] Hoffmann E, Dittrich-Breiholz O, Holtmann H and Kracht M. Multiple control of interleukin-8 gene expression. J Leukoc Biol 2002; 72: 847-855.

[28] Chen BC, Yu CC, Lei HC, Chang MS, Hsu MJ, Huang CL, Chen MC, Sheu JR, Chen TF, Chen TL, Inoue H and Lin CH. Bradykinin B2 receptor mediates NF-κB activation and cyclooxygenase-2 expression via the Ras/Raf-1/ERK pathway in human airway epithelial cells. J Immunol 2004; 173: 5219-5228.

[29] Shinkai M, Foster GH and Rubin BK. Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2006; 290: 75-85.

[30] Hiwatashi Y, Maeda M, Fukushima H, Onda K, Tanaka S, Utsumi H and Hirano T. Azithromycin suppresses proliferation, interleukin production and mitogen-activated protein kinases in human peripheral-blood mononuclear cells stimulated with bacterial superantigen. J Pharm Pharmacol 2011; 63: 1320-1326.

[31] Roe MFE, Bloxham DM, Cowburn AS and O'Donnell DR. Changes in helper lymphocyte chemokine receptor expression and elevation of IP-10 during acute respiratory syncytial virus infection in infants. Pediatr Allergy Immunol 2011; 22: 229-234.

[32] Kuo C, Lee M, Kuo H, Lin Y and Hung C. Azithromycin suppresses Th1- and Th2-chemokines. J Microbiol Immunol Infect 2019; 52: 872-879.

[33] Srivastava P, Vardhan H, Bhengraj AR, Jha R, Singh LC, Salhan S and Mittal A. Azithromycin treatment modulates the extracellular signal-regulated kinase mediated pathway and inhibits inflammatory cytokines and chemokines in epithelial cells from infertile women with recurrent Chlamydia trachomatis infection. DNA Cell Biol 2011; 30: 545-554.

[34] Kumar R, Khandelwal N, Thachamvally R, Tripathi BN, Barua S, Kashyap SK, Maherchandani S and Kumar N. Role of MAPK/MNK1 signaling in virus replication. Virus Res 2018; 253: 48-61.

[35] Ikegaya S, Inai K, Iwasaki H, Naiki H and Ueda T. Azithromycin reduces tumor necrosis factor-alpha production in lipopolysaccharide-stimulated THP-1 monocytic cells by modification of stress response and p38 MAPK pathway. J Chemother 2009; 21: 396-402.

[36] Willems-Widyastuti A, Vanaudenaerde BM, Vos R, Dilisen E, Verleden SE, De Vleeschauwer SI, Vaneylen A, Mooi WJ, de Boer WI, Sharma HS and Verleden GM. Azithromycin attenuates fibroblast growth factors induced vascular endothelial growth factor via p38MAPK signaling in human airway smooth muscle cells. Cell Biochem Biophys 2013; 67: 331-339.

[37] Speer EM, Dowling DJ, Xu J, Ozog LS, Mathew JA, Chander A, Yin D and Levy O. Pentoxifylline, dexamethasone and azithromycin demonstrate distinct age-dependent and synergistic inhibition of TLR- and inflammasome-mediated cytokine production in human newborn and adult blood in vitro. PLoS One 2018; 13: 1-29.