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Mechanism of azithromycin in airway diseases

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Abstract

Azithromycin (AZM) has been used to treat chronic inflammatory airway diseases because it regulates cell–cell contact between airway epithelial cells. Airway mucus hypersecretion is an important component of chronic respiratory diseases. Mucin 5AC (MUC5AC) is the major mucin produced by airway epithelial cells, and hypersecretion of MUC5AC is a sign of various pulmonary inflammatory diseases. Recently, it was found that matrix metallopeptidase 9 is involved in mucus hypersecretion. Moreover, AZM can inhibit the ability of TNF-α-to induce interleukin (IL)-8 production. This review focuses on the effects on AZM that may be beneficial in inhibiting MUC5AC, matrix metalloprotease-9 and IL-8 production in airway epithelial cells. In addition, recent studies have begun to assess activation of mitogen-activated protein kinase (MAPK) signaling pathways in response to AZM. Understanding these new developments may be helpful for clinicians.

Keywords

Azithromycin, MUC5AC, MMP9, IL-8, MAPK signaling pathway, chronic airway diseases

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Introduction

Azithromycin (AZM) is a widely used macrolide antibiotic with high tissue permeability and cell adhesion. AZM kills bacteria by reversibly binding to the bacterial ribosomal 50S subunit and inhibiting protein synthesis.[1] In addition to its antibacterial activity, AZM also plays an anti-inflammatory role by inhibiting secretion of pro-inflammatory cytokines including interleukin-8 (IL-8). IL-8 is an autocrine and/or paracrine tumor-promoting chemokine that regulates the survival and proliferation of various tumor cells. IL-8 is one of

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the first chemokines that activate neutrophils secreted by lipopolysaccharide (LPS)-stimulated monocytes.[2] First, neutrophil migration is induced, then a reticular structure is formed at the emission focus of chemokines. Reticulum formation plays a related role in inhibiting immune responses of neutrophils against tumors, which in turn is beneficial to the process of tumor metastasis.[3]

The abnormal expression of IL-8 in tumor cells affects metastatic potential through the production and secretion of matrix metalloproteinase 9 (MMP9).[4] AZM may suppress the activity and production of MMP9 in airway epithelial cells, inhibiting airway mucus hypersecretion. Moreover, AZM can inhibit IL-8 production induced by tumor necrosis factor (TNF)-α. Previous studies reported that AZM can inhibit the proliferation of cervical cancer and gastric cancer cells[5] and enhance the efficacy of chemotherapy against non-small cell lung cancer. However, the mechanism of the anticancer effect of AZM remains unclear.

Chronic airway diseases include chronic bronchitis, cystic fibrosis (CF), asthma, chronic obstructive pulmonary disease (COPD),[6] bronchiectasis and diffuse panbronchiolitis (DPB).[7] These diseases are primarily caused by chronic bacterial infections and mucus obstruction. Mucus secretion is useful to protect mucosal surfaces from pathogens and irritants.[8] However, in chronic airway diseases, excessive mucus secretion leads to airway obstruction and gas exchange disorders, both of which are important signs of airway diseases. Therefore, prevention of mucus hypersecretion is an important goal in treating chronic respiratory diseases. The major macromolecular component of mucus is mucin.[9] Among the 14 human mucoid genes identified to date, mucin 5AC (MUC5AC) encodes the major mucin core protein secreted from the airway surface epithelium.[10] MUC5AC is highly expressed in the lung and its expression was up-regulated by various bacterial stimuli.[11] A pathological increase in MUC5AC is characteristic of airway and mucus hypersecretion.[12] In recent years, many studies have reported that macrolide antibiotics can inhibit inflammatory mediators and play anti-inflammatory roles. Some studies showed that macrolides can directly inhibit secretion of MUC5AC by airway epithelial cells, potentially reflecting their potential as new anti-inflammatory drugs for the treatment of chronic sinusitis.[13] In the clinic, AZM has a significant inhibitory effect on airway mucus secretion.

AZM is also used to treat acute and infectious exacerbations of COPD.[14] Long-term use of low-dose AZM can prevent or delay deterioration of several respiratory diseases including asthma, COPD, CF and non-cystic fibrobronchiectasis. Other benefits include improved lung function in patients with diffuse panbronchiolitis and bronchiolitis obliterans syndrome as well as increased pulmonary function in patients with CF. Long-term low-dose treatment with AZM is associated with down-regulation of genes regulating antigen presentation, interferon and T cell responses, and multiple inflammatory pathways in the airway and blood of patients with neutrophilic COPD.[15]

In this paper, we comprehensively review mitogen-activated protein kinase (MAPK) (extracellular signal-regulated kinase [ERK] 1/2, $p38^{MAPK}$, and c-Jun N-terminal kinase [JNK]) signaling in response to bacteria, its role in stimulating the secretion and production of MUC5AC in human bronchial epithelial cells, and the effect of AZM on these signaling pathways. AZM can inhibit the activity and production of MMP9 in human bronchial epithelial cells, thus inhibiting airway mucus hypersecretion. Simultaneously, AZM can regulate ERK1/2 signaling and inhibit the

production of additional MMP9. Moreover, AZM can inhibit TNF-α-induced production of IL-8 via the JNK signaling pathway in human bronchial epithelial cells. Thus, our understanding of the role of macrolides in inhibiting mucus hypersecretion and production of IL-8 is progressively improving. AZM can inhibit MUC5AC production via the ERK1/2 and JNK signaling pathways, inhibit MMP9 production via the ERK1/2 signaling pathway and inhibit TNF-α-induced production of IL-8 via the JNK signaling pathway. The overall impact of these effects is to reduce mucus production as shown by several recent studies. Collectively, these data provide a theoretical basis for the use of AZM in the treatment of airway diseases, which may be helpful for clinicians.

Mechanism of AZM action on MUC5AC

Previous studies showed that the promoter of MUC5AC comprises two regions: an activator protein-1 (AP-1) binding site and a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) binding site.[16,17] Therefore, it is necessary to study the regulatory effect of AZM on both NF-κB and AP-1 mediated MUC5AC expression. Unlike NF-κB, which is mainly activated by the ERK1/2 and phosphoinositide 3-kinase (PI3K) Akt signaling pathways,[18] AP-1 is activated by ERK1/2, p38 ^{MAPK} and JNK by enhancing their downstream transcription factors including ETS Like-1 (Elk-1), c-Jun,[19] activating transcription factor (ATF)-2 and cAMP response element-binding protein (CREB). This regulates the expression of c-Fos and c-Jun, which are the components of the AP-1 complex.

NF-κB is a transcription factor, which plays important roles in inflammation and tumorigenesis. Previous studies have shown that various stimuli induce MUC5AC secretion by promoting NF-κB DNA binding activity.[20,21] ERK1/2, p38 MAPK, JNK, and PI3K Akt are the downstream signaling pathways of epidermal growth factor receptor (EGFR), which promotes expression of MUC5AC through the synergistic effects of NF-κB and AP-1.[22,23] It has been reported that AZM inhibits MUC5AC secretion by NCI-H292 cells in response to lipoproteins of *Haemophilus influenzae* at the mRNA and protein levels by selectively inhibiting the transcription factor AP-1.[17]

Acinetobacter baumannii, *Fusobacterium nucleatum* and *Pseudomonas aeruginosa* can stimulate airway epithelial cells to produce MUC5AC. Multidrug-resistant *A. baumannii* (MDRAB) induced MUC5AC production and gene expression.[24] The EGFR/ERK1/2/JNK-NF-κB pathways are involved in the production of MUC5AC in response to MDRAB. AZM inhibited MUC5AC expression induced by MDRAB. Therefore, AZM seems to reduce MUC5AC production by suppressing the phosphorylation of ERK1/2/JNK and nuclear translocation of NF-κB (Figure 1).

F. nucleatum induced MUC5AC production in airway epithelial cells via the ERK1/2 pathway. *F. nucleatum* induced airway epithelial cells to express MUC5AC at both the protein and the mRNA levels in both a time- and dose-dependent manner.[25] *F. nucleatum* induced phosphorylation of ERK1/2, but this was inhibited by AZM. Thus, AZM inhibited *F. nucleatum* induced MUC5AC production by suppressing the phosphorylation of ERK1/2 (Figure 1).

P. aeruginosa also induced airway epithelial cells to express MUC5AC at both the mRNA and the protein levels in a time and dose-dependent manner.[26] *P. aeruginosa* induced ERK1/2 and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (I-κB) phosphorylation, but this was inhibited by AZM. Hence, AZM can inhibit the production of MUC5AC in response to *P. aeruginosa* by suppressing the ERK1/2 pathway (Figure 1).

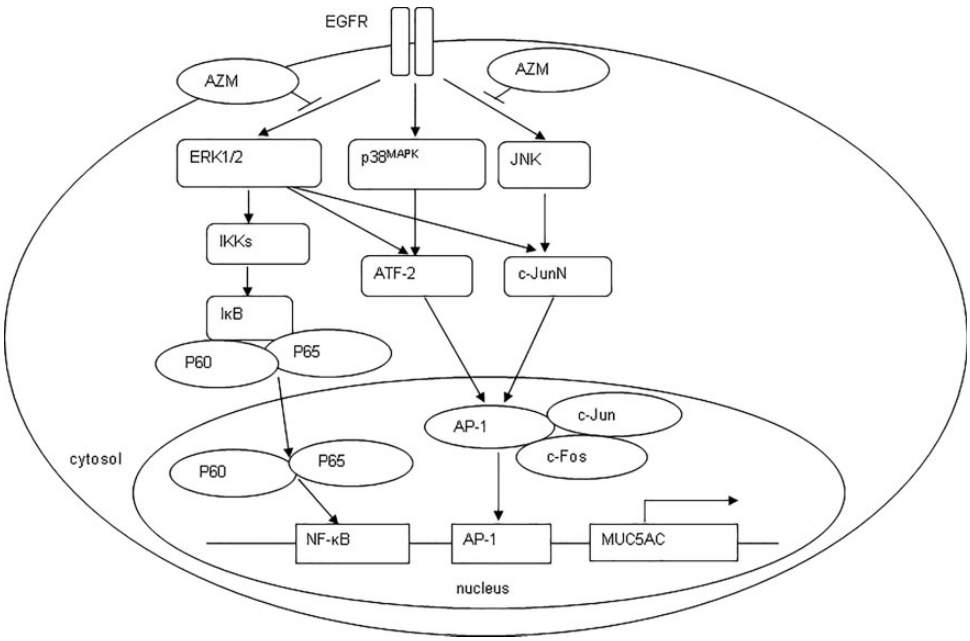

Figure 1. Proposed mechanism of AZM inhibition of bacteria induced MUC5AC secretion by airway epithelial cells. AZM suppresses the phosphorylation of ERK1/2 and JNK, which subsequently diminishes the activities of NF-κB and AP-1 via the IKKs/IκB/NF-κB and c-Jun/AP-1 signaling pathways. Inactivation of NF-κB and AP-1 reduces transcription of the MUC5AC gene.
AZM, azithromycin; MUC5AC, mucin 5AC; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; IKK, IκB kinase; AP-1, activator protein-1.

Mechanism of AZM action on MMP9

LPS, also known as endotoxin, is an outer membrane component of the cell walls of Gram-negative bacteria and is an effective activator of macrophages. *A. baumannii*, *F. nucleatum* and *P. aeruginosa* are all Gram-negative bacteria. In human bronchial epithelial cells, LPS may activate the ERK1/2 and JNK pathways, which in turn activate AP-1 activity and resulting in the expression of MMP9.[27,28] AZT can inhibit the overexpression of MMP9 and MUC5AC in airway epithelial cells stimulated with purulent airway secretions, and can also regulate the EGFR/ERK1/2 signaling pathways. Therefore, the EGFR/ERK1/2/JNK/AP-1 pathways are involved in bacteria-induced MMP9 production in human bronchial epithelial cells. AZM inhibits the production of MMP9 by inhibiting the phosphorylation of ERK (Figure 2).

MMP9, a member of the neutral protease superfamily, degrades basement membranes and the extracellular matrix, and participates in a variety of pulmonary inflammatory reactions.[29] Recently, it was found that MMP9 is involved in mucus hypersecretion. In addition, AZT can inhibit the activation of pro-MMP9 in human bronchial epithelial cells, increase the expression of tissue inhibitor of metalloproteinases (TIMP-1), and inhibit the activity of MMP9. Together these effects result in inhibition of airway mucus hypersecretion.

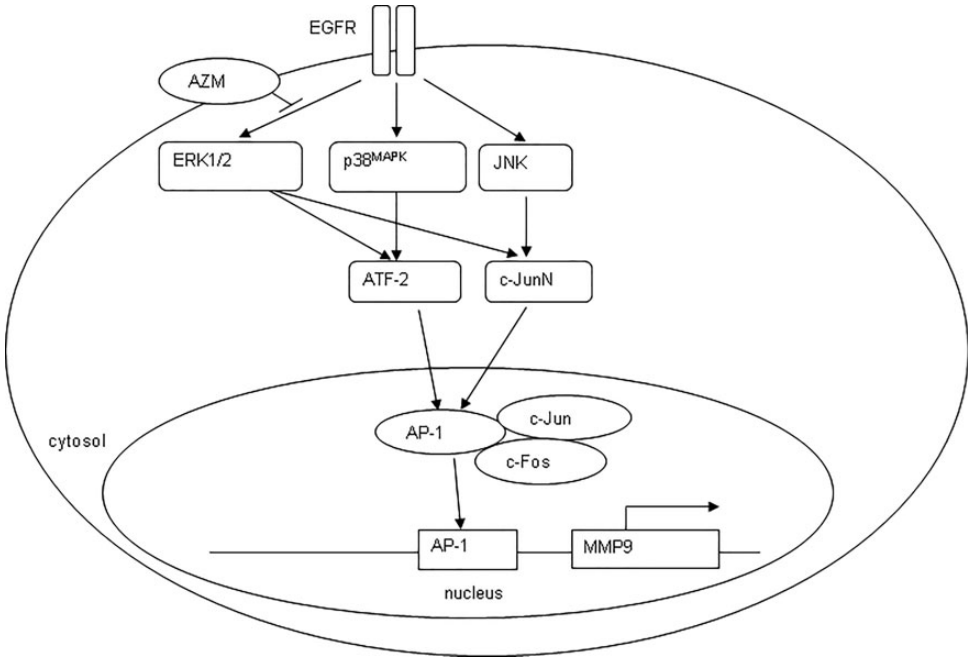

Figure 2. Proposed mechanism of AZM inhibition of bacteria induced MMP9 secretion by human bronchial epithelial cells. AZM suppresses the phosphorylation of ERK1/2, which subsequently diminishes the activity of AP-1. Inactivation of AP-1 reduces transcription of the MMP9 gene.
AZM, azithromycin; MMP9, matrix metalloproteinase 9; ERK, extracellular signal-regulated kinase; AP-1, activator protein-1.

Mechanism of AZM action on IL-8

IL-8 is one of the first chemokines that activates neutrophils secreted by LPS-stimulated monocytes. IL-8 is an autocrine and/or paracrine tumor-promoting chemokine that regulates the survival and proliferation of various tumor cells. The transcriptional level of IL-8 is regulated by many factors, including NF-κB, AP-1 and hypoxia-inducible factor (HIF)-1. Previous studies have shown that the EGFR-dependent MAPK/ERK1/2 signaling pathway was involved in TNF-α-induced IL-8 production in bronchial epithelial cells.[30]

Previous studies have shown that TNF-α stimulates the activation of JNK and p38 ^{MAPK} in human bronchial epithelial cells, but has no significant effect on ERK phosphorylation.[31] LPS did not induce phosphorylation of JNK and ERK, and phosphorylation of p38^{MAPK} was slightly increased. By contrast, the IκB kinase (IKK)-NF-κB signaling pathway plays an important and essential regulatory role in increasing IL-8 expression. The EGFR/MAPK/NF-κB/AP-1 pathway is involved in secretion of IL-8 induced by TNF-α. Hence, AZT can both suppress the activity of AP-1 in airway epithelial cells, inhibiting IL-8 production, and inhibit TNF-α-induced IL-8 production through JNK signaling in human bronchial epithelial cells (Figure 3).

Discussion

Mucus hypersecretion occurs in chronic airway diseases such as DPB and CF as

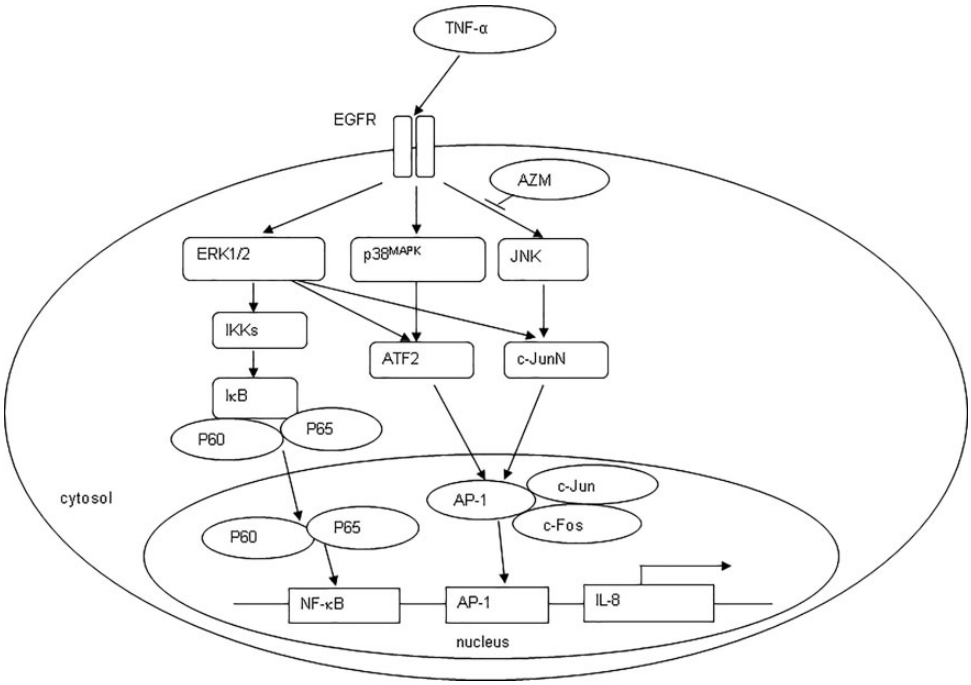

Figure 3. Proposed mechanism of AZM inhibition of TNF-α-induced MUC5AC secretion by human bronchial epithelial cells. AZM suppresses the phosphorylation of JNK, which subsequently diminishes the activity of AP-1 via the c-Jun/AP-1 signaling pathways. Inactivation of AP-1 reduces transcription of the IL-8 gene.
AZM, azithromycin; TNF, tumor necrosis factor, MUC5AC, mucin 5AC, JNK, c-Jun N-terminal kinase; AP-1, activator protein-1, IL, interleukin.

well as in ventilator-associated pneumonia (VAP). MUC5AC is the main core protein of mucins secreted at the airway surface epithelium. Therefore, control of mucus hypersecretion may be helpful for treatment of these diseases. VAP is the main cause of nosocomial infection-related death. VAP is difficult to treat because patients usually develop severe complications and often cannot tolerate invasive examinations. *A. baumannii* is the main pathogen causing VAP.[32] Carbapenems are recommended for empirical treatment of VAP associated with *A. baumannii*.[33] However, resistance of *A. baumannii* to carbapenems is becoming a serious problem. In addition, *A. baumannii* develops antibiotic resistance very rapidly.[34] MDRAB can induce MUC5AC production via the EGFR-ERK/JNK-NF-κB pathway, and AZM can inhibit MUC5AC expression induced by MDRAB. Thus, we speculate that macrolides may help to control VAP by reducing the amount of sputum.

Significant progress has been made in establishing the efficacy and safety of antibiotics for treatment of stable non-cystic fibrotic bronchiectasis. Oral AZM can slow the deterioration of the disease, while slightly improving quality of life and forced expiratory volume in the first second (FEV1). AZM can also improve FEV1 in patients with asthma.

F. nucleatum is a common anaerobic bacterium causing periodontitis, and also commonly causes anaerobic infection of the

respiratory tract.[35,36] *F. nucleatum* has pathogenic effects on airway epithelial cells. The products of *F. nucleatum* can induce MUC5AC production via ERK1/2 phosphorylation. It was also found that AZM and clarithromycin (CAM) inhibited MUC5AC production in response to *F. nucleatum*, while clindamycin (CLDM) and metronidazole (MTZ) had weaker effects. Periodontitis has been postulated to be associated with the pathogenesis of chronic respiratory tract infection. It was also suggested that macrolides can reduce the production of mucin, which may represent an additional therapeutic intervention with an independent mechanism from CLDM and MTZ.

P. aeruginosa is one of the most common pathogens responsible for chronic pulmonary infection.[37] Clinically, *P. aeruginosa* infection in the lung is often accompanied by excessive mucus secretion.[38] The supernatants of *P. aeruginosa* can upregulate the transcription of mucin genes.[37] LPS has been demonstrated to activate MUC5AC in supernatants of *P. aeruginosa* and rhinovirus can induce MUC5AC expression through the EGFR/ERK1/2/NF-κB pathway. AZM can inhibit the production of MUC5AC in response to *P. aeruginosa* by suppressing the ERK1/2 pathway. This provides a possible explanation for the clinical efficacy of macrolides for treatment of chronic respiratory *P. aeruginosa* infection.

Mycoplasma pneumoniae is a common cause of upper and lower respiratory tract infection, and typically manifests as pharyngitis, bronchitis and community-acquired pneumonia.[39–41] AZM, CAM and moxifloxacin (MXF) can effectively inhibit the growth of mycoplasmas and inhibit the production of IL-8 induced by TNF-α. However, none of them could inhibit IL-8 production in response to mycoplasma membrane fractions. *C. pneumoniae*, *Legionella pneumophila* and *H. influenzae* up-regulate NF-κB activation and increase MUC5AC production,[20,42] AZM, CAM and tirithromycin inhibited the production of MUC5AC in response to *C. pneumoniae* through the ERK and NF-κB signaling pathways.

MMPs play a critical role in COPD.[43] MMP9 is also called gelatinase B and has been confirmed to play a significant role in COPD. Some studies found that levels of MMP-9 in plasma were increased in type A1 trypsin deficiency emphysema and COPD.[44,45] LPS may activate the ERK1/2 and JNK pathways, enhancing AP-1 activity and resulting in MMP9 expression. AZM can inhibit the production of MMP9 by inhibiting phosphorylation of ERK in human bronchial epithelial cells. In additions, previous studies showed that *M. pneumoniae* infection can induce IL-8 expression by human epithelial cells and macrophages.[46,47] AZM, CAM and MXF can all inhibit the production of IL-8 induced by TNF-α, but none inhibited the production of IL-8 in response to mycoplasma membrane fractions.

Conclusion

The treatment of chronic airway diseases such as chronic bronchitis, asthma, COPD, DPB and CF is a major challenge. These diseases are characterized by mucus hypersecretion, increase of airway resistance and multidrug-resistant bacterial infections. Airway mucus hypersecretion is an important problem in chronic respiratory diseases. This review focused on the effects of AZM in chronic airway diseases: AZM can inhibit MUC5AC production via the ERK1/2 and JNK signal pathways, inhibit MMP9 production via the ERK1/2 signaling pathway, and inhibit TNF-α-induced production of IL-8 via the JNK signaling pathway. Collectively, these effects result in reduced mucus production as shown by recent studies. Together, these data provide a theoretical basis for the use

of AZM to treat chronic airway diseases, which may be helpful to clinicians.

Macrolides can inhibit mucus hypersecretion *in vivo* and *in vitro*. It is gradually being accepted that macrolides have both antibacterial activity as well as immunomodulatory effects. Recently, it was discovered that AZM plays a role in the treatment of chronic airway diseases. By inhibiting the activity of AP-1 and NF-κB, AZM can reduce levels of MUC5AC and MMP9, thus diminishing airway mucus hypersecretion. In addition, AZM can also inhibit TNF-α-induced production of IL-8 and exert an anti-inflammatory role. These data provide a possible explanation for the clinical efficacy of macrolides for treatment of chronic respiratory diseases.

Author contributions

Jie Yang conceived the study, wrote the article, searched the literature, and edited the article.

Declaration of conflicting interest

The author declares that there is no conflict of interest.

Ethics

This is a review of previously published articles. No human or animals were involved; therefore, no ethical approval was needed.

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