Research Paper

The Vitamin D - Vitamin D Receptor Axis Alleviates LPS - induced Acute Pulmonary Edema by Reducing Angiopoietin – 2

Xin Feng[1*], Yu Fu[2*], Bin-Cao Li[2], Juan Kong[2#]

[1] Liaoning Electric Porwer Center Hospital

[2]Department of Clinical Nutrition, Shengjing Hospital of China Medical University, Shenyang 110004, China,

*These authors have contributed equally to this work.

#Correspondence to: Juan Kong, **email:** kongj@cmu.edu.cn

Keywords: Pulmonary Edema, Tight junctions, Ang2, VD

Received: December 5, 2025 **Accepted:** March 22, 2025 **Published:** April 21, 2025

ABSTRACT

The mechanism by which the Vitamin D - Vitamin D Receptor Axis alleviates LPS - induced Acute Pulmonary Edema remains unclear, and the disruption of tight junctions may be one of the mechanisms. In this study, both animal and cell models were employed to investigate the possible reasons for the alleviation of acute pulmonary edema by vitamin D. The results demonstrated that VD indeed reduced the wet - to - dry ratio of the lungs, inflammatory cell infiltration, pathological symptoms, and the signaling pathways related to pyroptosis. Finally, it was discovered that VD alleviates pulmonary edema by inhibiting the disruption of tight junctions caused by Ang2 rather than directly antagonizing LPS.

INTRODUCTION

Vitamin D(1, 2), which cannot be independently synthesized in the human body and is sourced either through in - vivo synthesis upon skin exposure to ultraviolet rays or from the diet, regulates diverse physiological responses via its receptor VDR, including lipid, immune, and glucose metabolism, along with influencing various organs and animal behaviors. It functions in response to infections, warns of chronic diseases and cancers, and acts as an immunomodulator, participating in ALI - related pro - inflammatory cytokine expression and immune responses. Cholecalciterol Cholesterol Emulsion (CCE), a vitamin D analog (3, 4) and precursor of active vitamin D, can be converted into active vitamin D in the body to regulate calcium and phosphorus metabolism and bone formation.

pulmonary edema(5, 6), marked by abnormal fluid buildup in the lungs, is a grave condition that may culminate in respiratory failure and fatality. Among its diverse causes, LPS-induced pulmonary edema, closely linked to severe infections like sepsis, has garnered substantial attention. LPS, a component of Gram - negative bacteria's outer membrane, binds to toll - like receptor 4 (TLR4)(7, 8) on immune cells, resulting in the release of pro - inflammatory cytokines. The breakdown of these cellular connections

impairs the lung's normal barrier function(9), enabling fluid and proteins to leak from capillaries into alveolar spaces, thus leading to pulmonary edema. Understanding this mechanism is essential for devising effective treatments for this life - threatening condition(8).

Angiopoietin - 2 (Ang2)(10-13), a crucial regulator in the vascular system, has been found to have a significant impact on the integrity of tight junctions(14, 15), maintaining the normal barrier function of the pulmonary endothelium and epithelium, disrupted in the context of LPS - induced pulmonary edema. As a result, the permeability of the endothelial and epithelial barriers increases, facilitating the leakage of fluid and proteins into the alveolar spaces, which exacerbates the development of pulmonary edema. The complex relationship between Ang2 and tight junctions(16) underscores the importance of targeting this axis in the development of therapeutic strategies for LPS - induced pulmonary edema.

In this study, both animal and cell experiments were conducted to observe the effect of vitamin D (VD) in alleviating the impact of lipopolysaccharide (LPS) on pulmonary tight junctions via angiopoietin - 2 (Ang2).

MATERIALS AND METHODS

Construction of Animal Models and Grouping

A total of 36 male BALB/c mice were divided into four groups using the random number table method: the Control group, the CCE group, the LPS group, and the LPS + CCE group, with 9 mice in each group. Mice in the Control group and the LPS group were fed with drinking water, while those in the CCE group and the LPS + CCE group were fed with a cholecalciferol cholesterol emulsion aqueous solution (with a volume ratio of CCE to water of 1 μl:10 ml, protected from light). After 14 days of feeding, mice in the LPS group and the LPS + CCE group were instilled intratracheally with 10 mg/kg of LPS. Samples were collected 24 hours later, and bronchoalveolar lavage fluid was extracted to observe the wet - to - dry weight ratio of mouse lung tissue.

For the determination of the survival curve of mice, 20 healthy male BALB/c mice were selected, with 10 mice in each group. Mice in the LPS group and the LPS + CCE group were instilled intratracheally with 8 mg/kg of LPS, and the survival time of mice in each group was analyzed.

The impedance of tight junctions in cultured A549 cells is commonly measured using an Epithelial Voltohmmeter (EVOM) along with its corresponding electrode system, which is specifically designed for this purpose.

The transmission electron microscope was operated by professional technicians following the routine procedures, which were established based on standard practices in the field.

Both Western Blot, HE staining, and immunohistochemical staining were carried out in strict accordance with the routine operating procedures. All the antibodies utilized in these experiments were procured from the commercial market.

The SPSS 20.0 software was employed for the analysis of the results. The outcomes were expressed as mean ± standard deviation （X±S）. A difference was considered statistically significant when $P < 0.05$

RESULT

CCE effectively mitigated the LPS - induced pulmonary edema

Compared with the LPS group, CCE significantly reduced the weight of the lungs (Fig. 1A) and also decreased the wet - to - dry ratio of the lungs (Fig. 3B) in the CCE + LPS group with $p < 0.05$. However, CCE had no effect on blood calcium and phosphorus levels (Fig. 1B, C). Pathologically, CCE improved the cell infiltration in lung tissues and the degree of alveolar collapse (Fig. 2B), and thus significantly increased the survival rate of LPS - treated mice (Fig. 2A) by 20%.

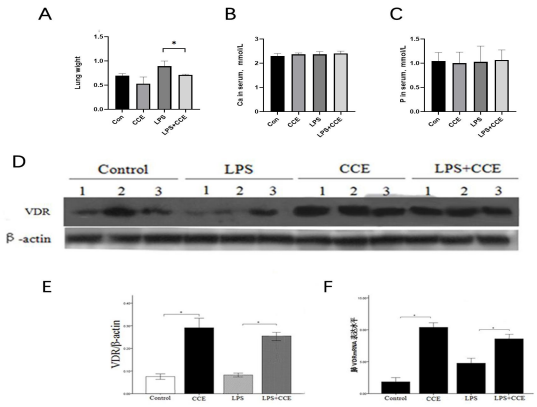

Figure 1: VD effectively mitigated the LPS - induced lung weight increase.A. Lung wight. B.Ca. C. P. D. Western of VDR. E,F. Quantification of VBR

CCE suppressed the generation of inflammatory factors and cell infiltration in alveolar perfusate.

Compared with the LPS group, the CCE + LPS group reduced the production of cytokine 6 in lung tissues and lung perfusate, as well as the infiltration of inflammatory cells (Fig. 3), and the pyroptosis - related factor Caspase - 1 (Fig. 4A, B, C). Moreover, electron microscopy revealed that the lung tissues in the LPS + CCE group had significantly intact cells and organelles, while the lung cells and organelles in the LPS group were swollen and damaged (Fig. 4D).

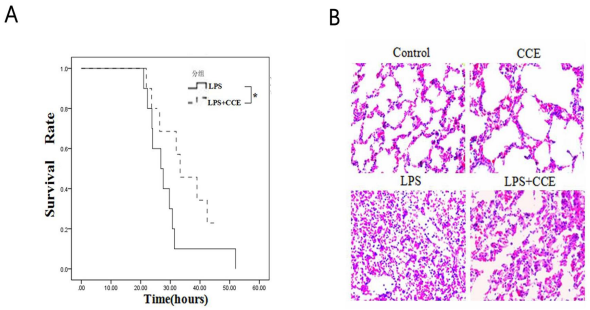

Figure 2: VD Reduces LPS - Induced Pathological Changes in the Lung and Mortality. A, Mortality; B. Pathology

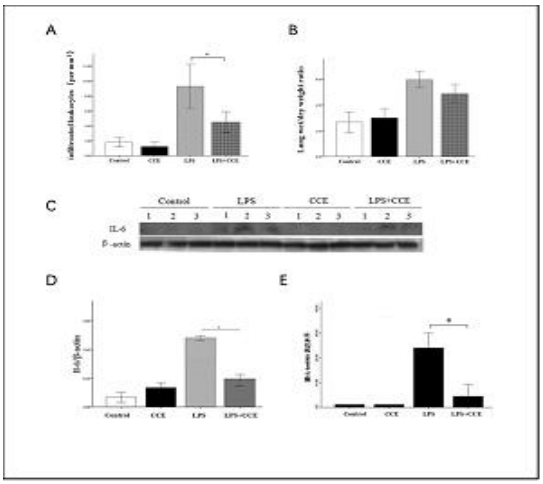

Figure3: VD diminishes the leukocyte infiltration and the levels of cytokines within lung tissues. A. Neutrophil in

bronchoalveolar lavage fluid (BALF) of mice, B. The wet/dry weight ratio of the lung. C. IL-6 Protein Levels in Lung Tissues D. Their Quantitative Analysis. E. The mRNA levels of IL-6.

CCE significantly augmented p - P38 and p - P42/44 levels in lung tissues.

Both Western blotting and mRNA analyses demonstrated that VD significantly augmented p - P38 and p - P42/44 levels in CCE + LPS cells compared with LPS cells （Fig.5）

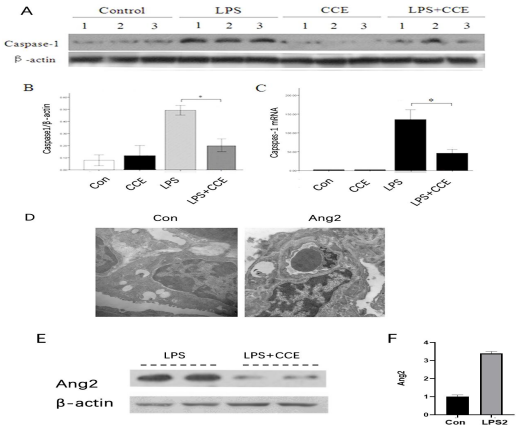

Figure 4: CCE alleviated cell pyroptosis and Ang2 (Angiopoietin - 2).

A. Western of Caspase-1, B. quantification of Caspase-1, C. mRNA of Caspase-1, D. Electron microscope of Pyroptosis E. Western of Ang2

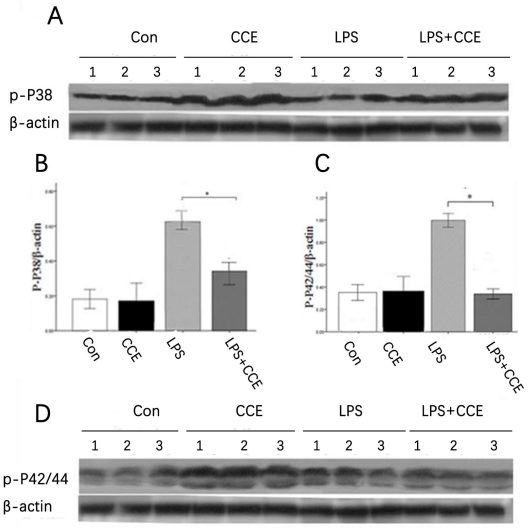

Figure 5: VD Significantly Increased p - P38 and p - P42/44 in Lung Tissues

A. Western of p - P38 protein B. quantification of p-P38 C. quantification p-P42/44 D. A. Western of p – P42/44， *p<0.05

CCE reduced Ang2 and enhanced tight junctions in lung epithelial cells.

CCE reduced the expression of Ang2 (Fig. 4E, F) in vivo and its nuclear translocation (Fig. 6C) in vitro. The enhancement of cell impedance by VD in Ang2 - related cells（Fig.6A）($p<0.05$) was significantly stronger than that in LPS cells (Fig.6B) at point of 15 hour marked by a blue arrow(Fig.6A,B), suggesting that the attenuation of edema and cell infiltration by VD in the LPS animal model may be mediated through inhibiting the expression and nuclear translocation of Ang2 rather than a direct effect of LPS.

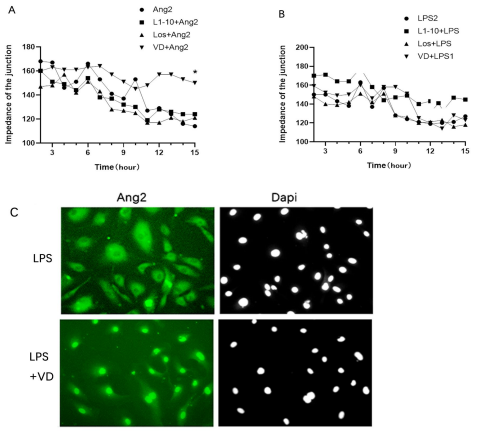

Figure 6: VD Augments the Impedance of Tight Junctions in Ang2 Cells, but Not in LPS Cells In Vitro

A. Impedance of Tight Junctions in Ang2 Cells, B. Impedance of Tight Junctions in LPS Cells, C. Immunohistochemistry of Ang2 nuclear translocation

DISCUSSION

To explore the impact of vitamin D on pulmonary edema, we administered lipopolysaccharide (LPS) to mice and closely monitored the state of lung effluent. Post - LPS treatment, the lungs exhibited an increase in weight, whereas vitamin D treatment led to a reduction in weight, as evidenced by both the wet - to - dry ratio and net weight measurements. This strongly suggested that vitamin D can effectively mitigate the development of pulmonary edema, as depicted in Figure 1 and Figure 3. Our results align with previous investigations (13, 17, 18); However, the conclusion of their research was attributed to the outcomes related to the renin - angiotensin system and the Ang2 - Tie2 axis (1).

Numerous studies(18, 19) have indicated that vitamin D alleviates pulmonary inflammatory responses, which is consistent with our current findings. Nevertheless, the underlying signaling pathways vary across different studies. Some research has implicated the NF - κB pathway(13), while others have focused on inflammatory cell infiltration. Our study revealed that the p38 and p42/44 pathways (Fig. 5) play a role in the regulation of renal inflammation by vitamin D.

Within the existing research framework, a complex and intricate relationship exists between the p - P38 and p - P42/44 pathways and Ang2. From a signal transduction perspective, p - P38 and p - P42/44 are integral members of the mitogen - activated protein kinase (MAPK) family(20, 21). In various cellular physiological and pathological processes, once activated by diverse upstream stimuli, these pathways are involved in governing crucial processes such as cell proliferation, differentiation, inflammatory response, and apoptosis. This study demonstrated that in the Vitamin D + LPS group, both the p38 and p42/44 path ways (Fig.5) were downregulated, accompanied by a corresponding decrease in Ang2 levels. This finding implies that vitamin D influenced the onset of pulmonary edema via these two signaling pathways.

To further elucidate whether the alleviating effect of vitamin D on pulmonary edema is a direct action on LPS or mediated through Ang2, we cultured A549 cells and measured the resistance of tight junctions. When vitamin D was added to Ang2 - treated cells, a notable increase in cell resistance was observed compared to LPS - only treated cells. Conversely, in the LPS + CCE - treated group, the increase in cell resistance was relatively modest and not statistically significant. As shown in Figure 6A and 6B, these results suggest that vitamin D may regulate LPS through the inhibition of Ang2 rather than a direct effect on LPS.

Immunofluorescence analysis of cells in the LPS + CC1 group revealed a significant nuclear translocation of Ang2. In contrast, in the LPS group, Ang2 was predominantly expressed in the cytoplasm (Fig. 6C). These findings further validate that vitamin D alleviates LPS - induced

pulmonary edema through modulating the expression and nuclear translocation of Ang2 rather than a direct effect of LPS, thereby strengthening the tight junctions in the lungs.

REFERENCES

1. Sizar O, Khare S, Goyal A, Givler A. Vitamin D Deficiency. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Swapnil Khare declares no relevant financial relationships with ineligible companies. Disclosure: Amandeep Goyal declares no relevant financial relationships with ineligible companies. Disclosure: Amy Givler declares no relevant financial relationships with ineligible companies.2025.
2. Dahash BA, Sankararaman S. Rickets. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Senthilkumar Sankararaman declares no relevant financial relationships with ineligible companies.2025.
3. Pan Y, Zhang Y, Liu N, Lu W, Yang J, Li Y, et al. Vitamin D Attenuates Alzheimer-like Pathology Induced by Okadaic Acid. ACS Chem Neurosci. 2021;12(8):1343-50.
4. Liu N, Li X, Fu Y, Li Y, Lu W, Pan Y, et al. Inhibition of lung cancer by vitamin D depends on downregulation of histidine-rich calcium-binding protein. J Adv Res. 2021;29:13-22.
5. Ahmad S, Zaki A, Manda K, Mohan A, Syed MA. Vitamin-D ameliorates sepsis-induced acute lung injury via augmenting miR-149-5p and downregulating ER stress. J Nutr Biochem. 2022;110:109130.
6. Houck P, Dandapantula H, Hardegree E, Massey J. Why We Fail at Heart Failure: Lymphatic Insufficiency Is Disregarded. Cureus. 2020;12(6):e8930.
7. Xue T, Zhang Q, Zhang T, Meng L, Liu J, Chai D, et al. Zafirlukast ameliorates lipopolysaccharide and bleomycin-induced lung inflammation in mice. BMC Pulm Med. 2024;24(1):456.
8. Mao Z, Lin X, Hu Y, Liu Y, Yu S, Zhou T, et al. Exploring the anti-inflammatory effects of Radix Curcumae essential oil in pulmonary sarcoidosis via the TLR4/MyD88/NF-kappaB pathway. Phytomedicine. 2025;139:156496.
9. Suresh K, Shimoda LA. Lung Circulation. Compr Physiol. 2016;6(2):897-943.
10. Gotts JE, Maishan M, Chun L, Fang X, Han CY, Chiueh V, et al. Delayed angiopoietin-2 blockade reduces influenza-induced lung injury and improves survival in mice. Physiol Rep. 2021;9(21):e15081.
11. Qiao J, Guo S, Huang X, Zhang L, Li F, Fan Y. Expression of Angiopoietin-2 in Lung Tissue of Juvenile SD Rats with Lipopolysaccharide-Induced Acute Lung Injury and the Role of Ulinastatin. Arch Immunol Ther Exp (Warsz). 2023;71(1):23.
12. Trent B, Liang Y, Xing Y, Esqueda M, Wei Y, Cho NH, et al. Polarized lung inflammation and Tie2/angiopoietin-mediated endothelial dysfunction during severe Orientia tsutsugamushi infection. PLoS Negl Trop Dis. 2020;14(3):e0007675.
13. Kong J, Zhu X, Shi Y, Liu T, Chen Y, Bhan I, et al. VDR attenuates acute lung injury by blocking Ang-2-Tie-2 pathway and renin-angiotensin system. Mol Endocrinol. 2013;27(12):2116-25.
14. Alharris E, Mohammed A, Alghetaa H, Zhou J, Nagarkatti M, Nagarkatti P. The Ability of Resveratrol to Attenuate Ovalbumin-Mediated Allergic Asthma Is Associated With Changes in Microbiota Involving the Gut-Lung Axis, Enhanced Barrier Function and Decreased Inflammation in the Lungs. Front Immunol. 2022;13:805770.
15. Aspal M, Zemans RL. Mechanisms of ATII-to-ATI Cell Differentiation during Lung Regeneration. Int J Mol Sci. 2020;21(9).
16. Zhou X, LeBleu VS, Fletcher-Sananikone E, Kim J, Dai J, Li B, et al. Vascular heterogeneity of tight junction Claudins guides organotropic metastasis. Nat Cancer. 2024;5(9):1371-89.
17. Yan Y, Lou Y, Kong J. MiR-155 expressed in bone marrow-derived lymphocytes promoted lipopolysaccharide-induced acute lung injury through Ang-2-Tie-2 pathway. Biochem Biophys Res Commun. 2019;510(3):352-7.
18. Xu J, Yang J, Chen J, Luo Q, Zhang Q, Zhang H. Vitamin D alleviates lipopolysaccharide‐induced acute lung injury via regulation of the renin‐angiotensin system. Mol Med Rep. 2017;16(5):7432-8.
19. Shi YY, Liu TJ, Fu JH, Xu W, Wu LL, Hou AN, et al. Vitamin D/VDR signaling attenuates lipopolysaccharide‐induced acute lung injury by maintaining the integrity of the pulmonary epithelial barrier. Mol Med Rep. 2016;13(2):1186-94.
20. Lavoie SS, Dumas E, Vulesevic B, Neagoe PE, White M, Sirois MG. Synthesis of Human Neutrophil Extracellular Traps Contributes to Angiopoietin-Mediated In Vitro Proinflammatory and Proangiogenic Activities. J Immunol. 2018;200(11):3801-13.

21. Neagoe PE, Dumas E, Hajjar F, Sirois MG. Angiopoietin-1 but not angiopoietin-2 induces IL-8 synthesis and release by human neutrophils. J Cell Physiol. 2012;227(8):3099-110.