

# Research Progress on the Mechanism of Action of Maxing Shigan Decoction in the Treatment of Viral Pneumonia

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**Abstract:** Viral pneumonia refers to the lung inflammation caused by a viral infection, the main causative agent is Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2), Influenza virus (Flu), parainfluenza virus (HPIV), adenovirus (Adv), respiratory syncytial virus (RSV), etc., Its main symptoms are fever, cough, shortness of breath, wheezing and wet lung rales, with changes in chest imaging (X-ray, ultrasound, CT, etc.), For the treatment of viral pneumonia, in addition to the treatment with influenza viruses that can be treated with neuraminidase inhibitors, no specific drugs for other viruses, Clinical mainly symptomatic treatment. Current studies have proved that the inflammatory cytokines IL-6, TNF- $\alpha$ ; the stimulating molecule CD137 and the chemokine CXCL-10, and the signaling pathway NF- $\kappa$ B signaling pathway, TLR 4-MyD 88-TRAF-6 pathway effectively inhibit inflammatory response, promote viral clearance, and effectively treat viral pneumonia. Viral pneumonia belongs to the category of "pneumonia asthma" in traditional Chinese medicine. For its heat syndrome and demonstration, such as wind heat, phlegm heat, toxic fever and other syndrome types, it can be used as the basic prescription for addition and subtraction treatment.

**Keywords:** Maxing Shigan Decoction; viral pneumonia; cytokines; stimulatory molecules; chemokines; signaling pathway

## 1. Introduction

Maxing Shigan Decoction are first seen in the "Treatise on Febrile Diseases" written by Zhang Ji, which is composed of ephedra, bitter almond, plaster and licorice, and the two share, both scattered wind heat, and heat and clear heat, and the weight of gypsum is more than ephedra, the two with plaster, lung asthma and no heat, gypsum with ephedrine, clear lung heat and not cool. Almond bitter temperature, and ephedra rise and fall, with gypsum clear coordination, is for the minister medicine, licorice gan ping, not only can prevent the gypsum bitter cold too much, but also can coordinate the drugs, for the medicine, four medicine xin warm cold and cool, a total of xin cool table, xuan lung flat asthma. Since its formation, Maxing Shigan Decoction has been widely used by doctors for its simplified formulation and remarkable efficacy.

## 2. Identification of inflammatory cytokines

### 2.1 IL-6

Interleukin 6 (IL-6) belongs to the interleukin family<sup>[1]</sup>, which induces a transcriptional inflammatory response mainly by binding to the interleukin 6 receptor  $\alpha$  (IL-6 Ra). IL-6 can be secreted by a variety of immune cells and stromal cells, such as macrophages, B cells, T cells and hepatocyte, adipocyte, dendritic cells, etc<sup>[2]</sup>. In the respiratory tract, IL-6 can also be produced by lung epithelial cells in response to the stimulation of allergens, respiratory viruses, and it has been shown that overexpression of IL-6 exists in bronchial epithelial cells of patients with pneumonia<sup>[3]</sup>. IL-6 is also involved in dendritic cell-T cell interactions to activate T helper cell 17 (TH17) cells; IL-6 binding to IL-6 Ra in hepatocyte promotes the production of C-reactive protein (C-reactive protein, CRP). CRP is an acute phase reactant that binds phospholipid components in damaged cells and microorganisms, and is often used as a high sensitivity

marker for infection and inflammation<sup>[4]</sup>. IL-6 also affect its pro-inflammatory vs. anti-inflammatory functions, IL-6 knockout mice showed different inflammatory response and increased susceptibility to infection caused by microorganisms<sup>[5]</sup>. The study of Wen Yang et al. Showed that in the rat model of severe pneumonia, serum IL-6 increased significantly compared with the blank group, and the group was significantly lower compared with the model group, suggesting that the group could effectively inhibit the excessive expression of IL-6 in the body.<sup>[6]</sup>

## 2.2 *TNF- $\alpha$*

Tumor necrosis factor-  $\alpha$  (tumor necrosis factor-  $\alpha$ , TNF-  $\alpha$ ), is a polypeptide cytokine, whose secretion is mainly derived from the activated monocyte-macrophages<sup>[7]</sup>, TNF-  $\alpha$  has multiple functions, such as mediating the expression of growth factors, other cytokines, transcription factors, and receptor genes, all of which are involved in the inflammatory process in response to pathogens<sup>[8]</sup>. During body inflammation, macrophages stimulate the production of vascular permeability factors by releasing TNF-  $\alpha$ , making it more prone to accumulate leukocytes<sup>[9]</sup>; stimulation of inflammatory factor interleukin 1 (IL-1) and chemokines, IL-1 can stimulate the expression of TNF-  $\alpha$  receptor and the generation of other cytokines, improve the sensitivity of TNF-  $\alpha$  in tissue cells, chemokines can induce a large number of leukocytes aggregation in the site of inflammation, and strengthen the clearance of pathogens in inflammatory sites<sup>[10, 11]</sup>. TNF-  $\alpha$  is essential for the cascade of cytokines<sup>[12]</sup>, it has been shown that once TNF-  $\alpha$  is inhibited, the levels of IL-6 and IL-1 will decrease within 12 hours<sup>[13]</sup>, Thus TNF-  $\alpha$  expression level are closely related to cytokine storm. Wang's study showed that for human lung fibroblasts cultured in vitro by type 3 Adv, the level of TNF-  $\alpha$  in the cell supernatant was significantly increased, while the decrease in the group was significant, and there was no significant difference from the ribavirin group<sup>[14]</sup>, the results showed that the high expression of TNF-  $\alpha$  and the severity of cytokine storm.

## 3. Stimulating molecules with chemokines

### 3.1 *CD137*

CD137, also known as 4-1BB, is a T lymphocyte costimulatory molecule, belonging to the tumor necrosis factor receptor superfamily (TNFRSF). The phosphorylation site of CD137 exists in T lymphocytes. When the body state is normal, the site is stable and the expression is not performed. When the body produces an inflammatory response, CD4<sup>+</sup>  $\square$  CD8<sup>+</sup>, NKT and other T lymphocytes are abnormally activated, and the CD137 phosphorylation site is activated with high expression. The high expression of CD137 can further promote the survival, expansion and differentiation of T lymphocytes<sup>[15, 16]</sup>, thus further affecting the immune-mediated function by T lymphocytes and enhancing the inflammatory response. It has also been shown that CD8 can be enhanced by the costimulatory effect of CD137<sup>+</sup>Or CD4<sup>+</sup>T cell expression to enhance the vaccine efficacy against influenza virus<sup>[17, 18]</sup>. The results of several studies show that when the body suffers from viral infection, TNFRSF including CD137 will show different degrees of high expression on T lymphocytes, which is closely related to the replication time of the virus. In the study of Chai Shaoqing et al., It could significantly reduce the CD137 expression level in the peripheral blood of mice with severe pneumonia, effectively inhibit the CD137 activation caused by lung inflammation, and correct the abnormal function of T lymphocytes, so as to reduce the inflammatory response and promote the recovery of the body<sup>[19]</sup>.

### 3.2 *CXCL-10*

CXCL-10 belongs to the CXC chemokine family. It is a small molecule protein that acts as a chemoattractant in the migration of immune cells in response to infections caused by viruses or microorganisms. CXCL-10 is mainly induced by TNF-  $\alpha$ , IFN-  $\gamma$ , and viral RNA, which is a membrane-spanning G protein-coupled receptor<sup>[20]</sup>, It is mainly expressed on leukocytes, endothelial cells, mononuclear macrophages and other cells. After CXCL-10 binds to its receptor, it can chemotize the aggregation of T lymphocytes, monocytes and natural killer cells and promote the secretion of cytokines such as IL-6 and IFN-  $\gamma$ <sup>[21]</sup>. In the lipopolysaccharide (LPS) -induced acute respiratory distress syndrome (ARDS) murine lung injury model, CXCL10 expression was significantly up-regulated, and the neutralization of CXCL10 with anti-CXCL10 antibodies promoted the improvement of lung injury<sup>[22]</sup>. Several studies have shown a correlation between CXCL10 levels in serum, alveolar lavage fluid and the severity and duration of acute respiratory infections due to viral infection<sup>[23]</sup>, Even in COVID-19, it was positively associated with an increased risk of patient mortality. Wang human research showed that in

the mouse model of pneumonia caused by influenza virus, in addition to the blank control group, each group of CXCL10 present high expression state, after the hemp apricot soup and oseltamivir administration after 3d, the CXCL10 levels decreased significantly, and there is no significant difference between the two, in the experiment, hemp apricot soup in addition to inhibiting chemokines, promote inflammatory infiltration absorption, can also increase the number of beneficial lung flora, improve lung micro ecological environment, it may also be for inflammation caused by lung damage played a recovery effect<sup>[24]</sup>.

#### 4. Signaling pathway

##### 4.1 The NF- $\kappa$ B signaling pathway

Nuclear factor  $\kappa$  B (NF- $\kappa$ B) is an important mediator of the inflammatory response and the immune response, When inflammation occurs, Inflammatory factors such as TNF- $\alpha$ , IL-1 bind to their related receptors, Activof I  $\kappa$  B kinase (Inhibitor of I $\kappa$ B Kinase, The IKK) complex thus causes the phosphorylation of the p65 and NF- $\kappa$ B inhibitory protein (I  $\kappa$  B  $\alpha$ ), Also induced the transfer of p65 from the cytoplasm into the nucleus, And then initiate the related inflammatory factors, Improve the inflammatory response in the body, After the activation of the NF- $\kappa$ B signaling pathway, Its nuclear morphology is usually active within an hour, But it can peak in just over few minutes<sup>[25, 26]</sup>. A study showed that in ovalbumin (ovalbumin, OVA) -induced airway inflammation in mice, airway infiltration and mucus production and decreased immunoglobulin and T helper cell 2 (Th-2) cytokine expression<sup>[27]</sup>. Some studies showed that in the rat model of severe pneumonia, the protein expression of I $\kappa$ B $\alpha$  in rat lung tissue was significantly reduced, and the expression of p65 and phosphoryl factor  $\kappa$  B inhibitor (p-I $\kappa$ B $\alpha$ ) was significantly increased; and the related substances of NF- $\kappa$ B signaling were significantly improved after treatment<sup>[6]</sup>.

##### 4.2 The TLR 4-MyD 88-TRAF-6 pathway

TLR 4 is a typical pattern recognition receptor (PRRS) in the Toll-like receptor family (Toll-like receptors, TLRs). It is the main receptor for gram-negative bacteria lipopolysaccharides (LPSs), and also recognizes the surface glycoproteins of RSV, IFV and other enveloped viruses, which is highly expressed on the surface of monocyte-macrophages<sup>[28]</sup>. When the virus invaded the body, the virus itself and its envelope protein will continue to stimulate TLR 4, TLR 4 directly or indirectly through PRRS, through the connecting molecule MyD 88 will signal to the cells, start the MyD 88-dependent signaling pathway, activate tumor necrosis factor (TNF) receptor related protein 6 (TRAF 6), and then cause IL-1  $\beta$ , TNF- $\alpha$  and other pro-inflammatory factors, mediate the body to produce immune response, cause cytokine storm in the body, leading to inflammatory response<sup>[29]</sup>. It has been shown that pneumonia due to IFV is closely associated with a progressive inflammatory response during IFV infection, and the activation of TLR 4 aggravates the pulmonary inflammatory response triggered by IFV<sup>[30]</sup>, the study of his Guliang et al. Showed that the use of decoction can effectively inhibit the overexpression of this pathway and proinflammatory factors in the animal model of influenza virus induced pneumonia, and reduce the lung inflammatory response caused by it<sup>[29]</sup>.

#### 5. Discussion

Maxing Shigan decoction curative effect is significant in clinical use, for pneumonia of fever and cough in the two main symptoms, hemp apricot stone sweet soup has a good effect, in the animal fever model induced by beer yeast, gypsum and licorice respectively by reducing the hypothalamic prostaglandin E<sub>2</sub> level and effect on the pituitary adrenal axis play the role of antipyretic<sup>[31, 32]</sup>. The decoction can dilate bronchial smooth muscle by inhibiting histamine and acetylcholinergic receptors in the bronchus. The ephedrine in ephedra activates the  $\alpha$  and  $\beta$  adrenoceptors, and the amygdalin in bitter almond can inhibit the cough center, so as to achieve the purpose of relieving cough<sup>[33]</sup>. Its is widely used in the treatment of pneumonia caused by various reasons, whether viral pneumonia, or bacterial pneumonia, after accurate dialectical symptomatic medication, Maxing Shigan decoction has a good curative effect, Li Shanshan and others use with Maxing Shigan decoction treating children with mycoplasma pneumonia, found that it can effectively inhibit the body inflammatory reaction, improve immune function, and can improve the lung function<sup>[34]</sup>. it has also played an important role in the prevention and treatment of COVID-19, among the typical representatives of "three drugs and three parties" formed in the clinical practice of fighting against COVID-19, in addition to the Xuebijing

injection solution, Lianhua Qingwen capsule, Jinhua Qinggan granules, Qingfei detoxification soup, changes wet poison prescription, and Xuan lung poison prescription are all from Moxing Shigan decoction as the main side and cut, clinical use effect proves that it can effectively relieve the typical clinical symptoms of patients, accelerate the removal of the virus, reduce the inflammatory response and reduce the occurrence of complications, It also plays a preventive effect, Among the confirmed COVID-19 cases in China, the overall utilization rate of traditional Chinese medicine reached 91.5%, total response rate was greater than 90%, Moxing Shigan decoction plays an important role in it.

However, there is still some controversy about the safety of the active ingredients in the decoction. Some studies believe that the ephedrine alkaline active ingredients can stimulate the cardiovascular and central nervous system and lead to adverse reactions. Amygdalin can be converted into cyanide, which may lead to cyanide poisoning, but LIN<sup>[33]</sup> On subacute toxicology experiments showed that in the LPS induced rat model, five times of the effective dose of 28 days, the experimental group of rats blood biochemical index, liver and kidney work, urine analysis and other laboratory indicators and control group is no statistical difference, the main organ histopathology is no abnormalities.

Although the decoction are widely used, but the research on its modern pharmacology is still not deep enough, especially for its mechanism of action within the organism, about the production mechanism of inflammation is a very complex process, the current experimental designed pathways is relatively simple, and for the quantity and effect relationship of the Moxing Shigan decoction, only very few studies have addressed so far, not only the number of experimental samples was small and there was no ultra-high dose group, regarding the peak of potency at dose escalation remains unknown, its toxicology is remains on the surface, the mechanism of action of its possible adverse components in vivo remains unknown, interaction between drugs is also poorly studied. Therefore, based on the optimization rat model, the mechanism of action from large sample, multiple pathway, multiple target, toxicology, and pharmacology research will be the focus of future research.

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