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The mechanism of action of traditional Chinese medicine in the prevention and treatment of influenza

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Abstract. Influenza, because of its virus is highly contagious and easy to mutate, has caused many outbreaks around the world. Traditional Chinese medicine (TCM) has been used in China for the prevention and treatment of influenza since ancient times, and now more and more people choose to use TCMs to prevent and treat influenza in their daily life. At present, researchers have also tried to study the mechanism of action of TCMs against influenza virus from multiple angles, but there are still many problems in the existing research that deserve further improvement. This article summarizes the researches on the mechanism of action of various TCMs against influenza virus by researchers at home and abroad in recent years and divides the mechanism of action of TCM against influenza virus, which according to the pathogenic mechanism of influenza virus, into two categories: direct inhibition and indirect inhibition. At the same time, this article found that many TCMs can also reduce the damage caused by viral infections by inhibiting the body's excessive immune response. This article also found that there are still some problems in the current research, such as unclear active ingredients, unclear effective dosage, lack of clinical trials, lack of comparative experiments between Chinese and Western medicines, and lack of research on influenza virus B, etc., which are hoped to be focused on in the future researches.

Keywords: Influenza, influenza virus, Traditional Chinese medicine, Mechanisms.

1. Introduction

Influenza is an acute respiratory infection caused by influenza virus. Influenza virus is highly contagious and easy to mutate, making the population generally susceptible and have caused many outbreaks around the world. Modelling estimates that between 300,000 and 650,000 people die each year from respiratory problems caused by influenza [1], which poses a serious threat to global human health.

Clinically, Influenza can be divided into four types: simple, gastrointestinal, pneumonic and poisonous. Common symptoms include fever, chills, headache, fatigue, loss of appetite, muscle aches and other systemic manifestations, gastrointestinal Influenza, which are more common in children, can also have gastrointestinal symptoms such as vomit and diarrhea. Patients with pneumonia will have critical symptoms such as high fever and hemoptysis, and the mortality rate of this type is high, and ultimately death is mostly due to respiratory and circulatory failure within 5-10 days. Systemic toxemia is present in patients with the poisonous form, and this type has a high mortality rate but is rare.

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The influenza virus is prone to antigenic drift and antigenic switch, which makes the development of influenza vaccines very difficult, making it impossible for people to obtain long-term immunity to influenza virus. At present, Western drugs commonly used in clinical treatment for influenza include Amantadine and Oseltamivir. Amantadine has now been found to be basically resistant to influenza virus and has been rarely used in clinical practice. Oseltamivir can specifically inhibit the neuraminidase of influenza virus, but it has a series of adverse reactions, so its use is also limited in clinical practice.

In the theory of TCM, influenza belongs to the category of "wen disease" (plague disease), which is caused by the “yi qi” (epidemic evil), with rapid onset, rapid transmission, and more serious symptom. This disease is often treated with the TCMs, which is heat-clearing and antidote. Since ancient times, there have been many records of TCM in the prevention and treatment of influenza in China, and all of them have good curative effects. A large number of TCMs are still being used in prevention and treatment of influenza in people's daily lives. The sales of the Chinese patent medicine products for the treatment of colds rose remarkably, and the sales of Lianhuaqingwen Granules had the sharpest jumps during these years, and many new Chinese patent medicine drugs are also on the way to research and development. This shows that more and more people will choose TCM to prevent and treat colds in their daily lives.

Undoubtedly, TCMs have great effects on the prevention and treatment of influenza, but their specific mechanism of action is not clear, this article summarizes the mechanism of some TCMs, which is commonly used in clinical to intervene influenza, and puts forward the shortcomings in the current research, hoping to provide some reference for future research.

2. The pathogenic mechanism of influenza virus

Influenza virus belongs to the family of Orthomyxoviridae, is a single-negative-strand RNA virus, and its basic structure includes a nucleocapsid and an envelope outside the nucleocapsid, and its structure is shown in Figure 1. The nucleocapsid of the influenza virus is composed of viral RNA, RNA polymerase, and nucleoprotein (NP). Together, NP and viral RNA make up the ribonucleoprotein (RNP). The envelope of influenza virus is a two-layer structure, with matrix protein (M1) on the inner layer and lipid bilayer structure on the outer layer from the host cell membrane. The envelope of influenza virus A and B is embedded with three membrane proteins: hemagglutinin (HA), neuraminidase (NA) and membrane protein (M2). HA and NA make up the spikes on the surface of the influenza virus, and M2 is embedded in the envelope as a transmembrane protein.

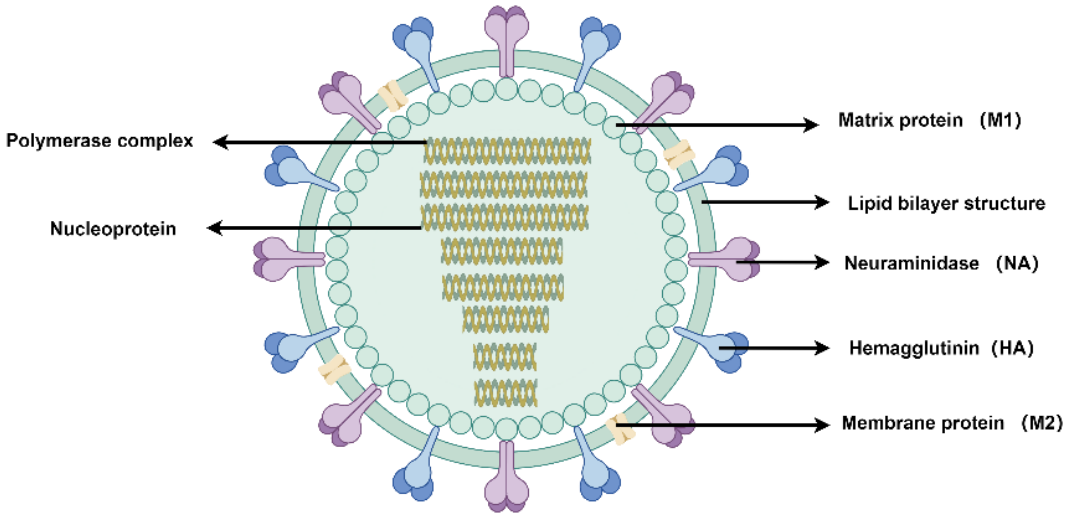

Figure 1. The structure pattern diagram of Influenza virus (By Figdraw).

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3. The pathogenic process of influenza virus

Influenza virus is mainly inhaled through the respiratory tract and then attacks humans, and their pathogenic process is shown in Figure 2. Viral surface proteins HA specifically recognize and bind to host airway epithelial cell surface receptors to complete virus adsorption. Influenza viruses fuse with host cells through the envelope to mediate the endocytosis of virions and complete the penetration of the virus. The virus releases RNP to finish uncoating itself once it has entered the cell and destroys the protein capsid. In the host cell nucleus, the viral RNA completes transcription and replication—a process known as biosynthesis. It then enters the cytoplasm for translation and assembles into new influenza virus proteins. The influenza virus further develops, acquires an envelope, and forms spikes on the surface of the envelope, completing the process of maturation of the virus. Finally, with the help of NA and M1 proteins, the virus is released from the cell in the form of budding, and then invades other airway epithelial cells, causing the diffusion. It causes cell degeneration, necrosis, and shedding, resulting in local inflammation and even systemic toxicity.

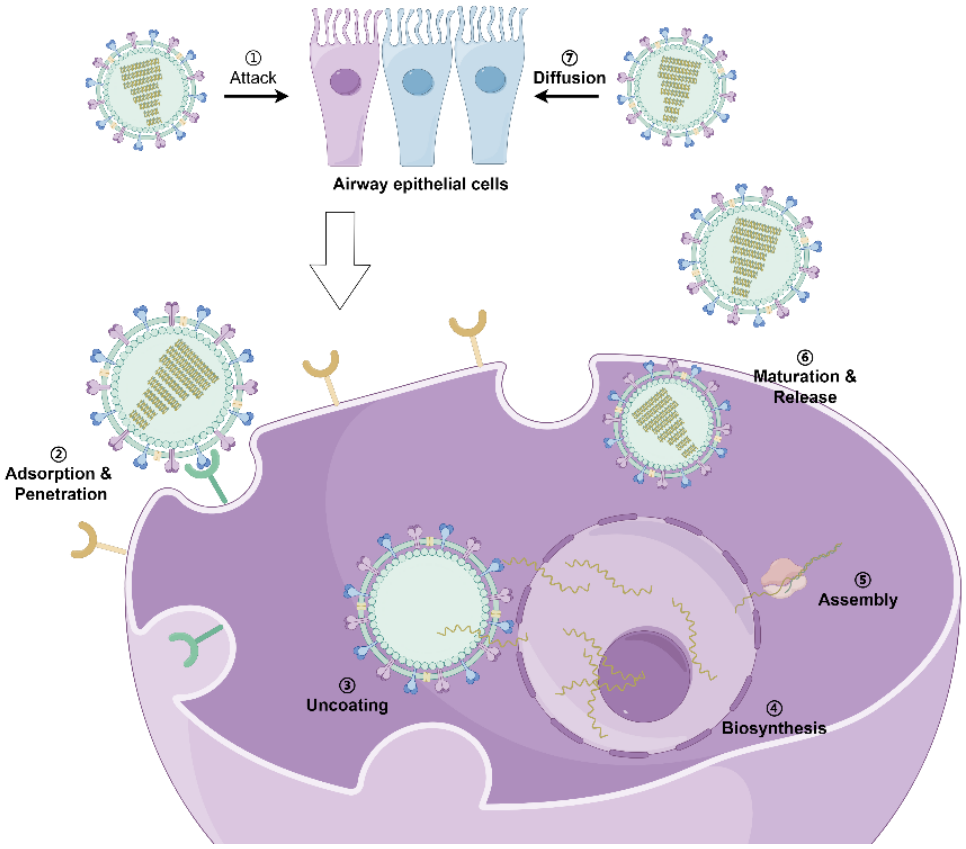

Figure 2. The pathogenic process of influenza virus (By Figdraw).

4. The mechanism of action of TCM in the prevention and treatment of influenza

By searching the relevant literature on the prevention and treatment of influenza with TCM in recent years, and summarizing the mechanism of action of TCM in the prevention and treatment of influenza mentioned in the article, this article found that the mechanism of action can be divided into two categories: direct inhibition and indirect inhibition. Direct inhibition means that the drug acts directly on the influenza virus to block the proliferation process of the virus. According to the pathogenic process of the influenza virus mentioned above, it can be found that in the process of virus proliferation, the virus surface protein HA, the virus surface protein NA and the contents of the virus nucleocapsid play

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great roles, so this article subdivides direct inhibition into three categories: inhibition of influenza virus surface protein HA, inhibition of influenza virus surface protein NA and inhibition of influenza virus nucleocapsid contents. Meanwhile, some literature also put forward some uncommon mechanisms, such as inhibition of autophagy and apoptosis of influenza virus, inhibition of the expression of influenza virus M1 protein, and inhibition of the activity of enzymes required for influenza virus replication, etc., which will also be summarized in this article.

When the influenza virus invades the body, it will attack the airway epithelial cells in the human body. These cells that die due to viral infection will release relevant cytokines, such as interleukins (IL) and interferons (IFN) , etc., before apoptosis, and summon macrophages, neutrophils, etc. to phagocytosis the virus through their concentration differences, and can also summon natural killer cells (NK cells) to directly kill target cells, so as to complete the body's non-specific immunity, at the same time, T cells and B cells will also produce antibodies under the series of effects of cytokines to complete the body's specific immunity. Therefore, indirect inhibition refers to the drug acting on a series of cytokines and immune pathways, activating various immune cells, reducing the damage of the virus to the body, and indirectly achieving the purpose of antiviral. Compared with direct inhibition, indirect inhibition is less likely to produce resistance to influenza virus.

At the same time, studies have shown that the pathological damage caused by influenza infection is not the direct damage caused by the virus itself, but the excessive inflammatory response produced by the body, that is the "cytokine storm". In severe cases, this can cause acute lung injury and severe respiratory distress syndrome, which is widely recognized as a main reason of pathological injury and death in influenza infection process [2]. The preventive and therapeutic effect of TCM on influenza is more significantly reflected in this part that inhibit the virus-induced inflammatory response, and alleviate the excessive immune response produced by the body. Therefore, this article will also further analyze the TCM that can effectively inhibit the body's excessive immune response and its specific mechanism of action mentioned in the literature in recent years.

4.1. Direct inhibition

4.1.1. Inhibition of influenza virus surface protein HA. The commonly used TCM to inhibit HA in TCM prescriptions are Poncirus trifoliata and Radix isatidis. A study focused on the extract of Poncirus trifoliata seed showed that the seed ethanol extract of Poncirus trifoliata can inhibit the HA-mediated viral adsorption and penetration process of influenza virus by preventing the attachment and acidification of the virus, thereby inhibiting the reproduction of influenza virus, especially oseltamivir-resistant strains, in MDCK cells [3]. According to Li et al.'s findings, some in-vitro tests has shown that the aqueous extract of Radix isatidis effectively inhibited the H7N9 virus. They hypothesized that its inhibition mechanism was to prevent the influenza virus HA from adhering to host cells, which in turn prevented the virus from entering host cells to continue replicating [4].

4.1.2. Inhibition of influenza virus surface protein NA. Currently, effective drugs commonly used in TCM to inhibit NA are Honeysuckle, Skullcap and Semen Sojae Praeparatum. Li et al. showed that the antiviral active ingredients in Honeysuckle were caffeic acid and chlorogenic acid, and it had the effect of inhibiting influenza virus NA at doses ranging from 0.2 to 2000 μg/mL in vitro canine kidney cell experiments, and there was a dose-response relationship [5]. Yue Ding's study showed that chlorogenic acid can inhibit NA in cells and animal models, thereby inhibiting the release and spread of influenza virus A, especially H1N1 and H3N2 [6]. At the same time, this study showed that the NA of influenza virus A could effectively be inhibited by the baicalin in Skullcap in a dose-dependent manner in canine kidney cell models and mouse models [7]. Su Zhen-Zhen's study showed that baicalin had a stronger inhibitory effect on H3N2 than H1N1 [8]. Research using 2 mg/mL Douchi effectively inhibited 80% of NA activity, thereby blocking the release of progeny viruses, confirming the effectiveness of Douchi. Although its inhibitory strength is not as strong as oseltamivir, it has higher safety [2].

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4.1.3. Inhibition of influenza virus nucleocapsid contents. Existing studies have shown that the commonly used TCMs that inhibit nucleocapsid contents are Melia Fructus, Dandelion and Honeysuckle. Young's study showed that the main active ingredient of Melia Fructus has a higher affinity for binding to PA protein than the known PA protein inhibitor R05-01, which mainly prevents the reproduction of the virus by altering the nuclear localization of PA protein in the early stage of viral replication, disrupting nuclear transport, leading to cytoplasmic accumulation of PA protein, and inhibiting the replication of influenza virus mRNA [9]. A series of in-vitro experiments have shown that extracts of Dandelion can inhibit the multiplication process of the virus by inhibiting the synthesis of nuclear proteins of H1N1 virus in canine kidney cells or human lung adenocarcinoma cells, thereby inhibiting the reproduction process of the virus [10]. Li's research showed that miR2911, a stable antiviral ingredient in Honeysuckle decoction, targets the PB2 and NS1 proteins of H1N1, H5N1, and H7N9 influenza viruses, thereby inhibiting the replication of influenza viruses [11].

4.1.4. Others. Because viruses evade host attack by regulating autophagy and apoptosis, which is one of the main ways for viruses to support their own effective replication and infection, so inhibiting these two pathways can also play a certain antiviral role. Zhu's study showed that baicalin in Skullcap can effectively inhibit autophagy induced by H3N2 influenza virus in A549 cells and Ana-1 cells [12]. Wu Tong's study showed that baicalin alleviates lung pathological damage in mice infected with H1N1 virus by downregulating endoplasmic reticulum stress response and inhibiting apoptosis of histiocytes [13]. Anna Hing-Yee Law's study showed that forsythoside A in Forsythia can limit the spread of the virus by inhibiting the expression of the influenza virus M1 protein, and the study also speculated that Forsythia may inhibit the synthesis of viral proteins by inhibiting the activity of enzymes required for viral replication, thereby acting as an antiviral [14].

4.2. Indirect inhibition

It is mainly through the way of improving the body's immunity, and then playing a role in fighting viral infection. Among the TCMs, medicines commonly used to indirectly inhibit influenza viruses are Skullcap, Radix isatidis and Schizonepeta tenuifolia Briq. Ming's research has shown that baicalin in Skullcap can enhance immunity by inducing the production and release of IFN-γ in T cells and NK cells [15]. Wen Song et al. showed that α-glucan in the Radix isatidis can significantly promote the proliferation of T cells and B cells in the spleen of the mice which is immunized with H1N1 influenza vaccine, and can also promote the secretion of IFN-γ and IL-4 by spleen and thymocytes, indicating that α-glucan has a good effect on the humoral immunity and cellular immunity [16]. Tang's research showed that the volatile oil of Schizonepeta tenuifolia Briq has a good anti-inflammatory effect, and in chicken embryo experiments, it can increase the levels of IFN-α, IFN-β, and IL-2 in serum, and reduce the levels of IL-6 and TNF-α, thereby enhancing the immune regulation function of the body after influenza infection and alleviating the lung damage caused by influenza virus [17].

4.3. Inhibition of excessive immune response

Andrographis paniculata and Radix isatidis are often used to inhibit the body's excessive immune response. Chen's research has shown that Andrographis paniculata is a natural antibiotic drug, and andrographolide can reduce viral load and inflammatory cytokine expression by participating in the activation of NF-κB, Nrf2, JAK-STAT, and RLRs signaling pathways, and ultimately alleviate the damage caused by cell death caused by influenza virus infection [18]. Li's study showed that lignin in the Radix isatidis can slow down the activation of the NF-κB signaling pathway in MDCK cell experiments, and can inhibit the expression of H1N1 virus-induced inflammatory cytokines. In addition, it also can reduce the expression of IP-10 and TNF-α induced by H9N2 virus [19]. Chris' study showed that indirubin and its derivatives in the Radix isatidis can significantly inhibit the excessive release of pro-inflammatory cytokines, such as IP-10, which is one of the key factors in lung inflammation caused by H5N1 virus. The study also showed that the indirubin and its derivatives could be one of the drugs for patients with severe H5N1 virus infection [20].

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5. Discussion

As a treasure of China, the TCM is a diverse and inexpensive resource. According to several groups of data, the application of Chinese medicine in influenza prevention and treatment is extensive and has long-term research and development prospects. However, this article finds that there are still many problems in the existing studies that deserve improvement in future research.

5.1. The active ingredients are not yet clear

Currently, most studies on the treatment of influenza with Chinese medicines focus on the pharmacological effects of the drugs, but the determination of the pharmacodynamic substances involved is lacking. TCMs are multi-component and multi-targeted. Specifically, a single Chinese medicine may have multiple pharmacological effects. Therefore, in the subsequent studies, some high-precision instruments can be used to extract and separate their components, screen out the effective pharmacodynamic substances, and further study the mechanism of action.

5.2. The effective dose is not yet clear

Most research results did not indicate the exact effective dose of their research substances. Hence, subsequent research can focus more on determining the effective dose of the active ingredient, which can help clinical workers to develop a safer, more effective and better dosage regimen.

5.3. Lack of clinical trials

The major research methods in this direction include in vitro cellular experiments, chicken embryo experiments and in vivo experiments in animals. Many medicines are only effective in in vitro experiments, but their clinical efficacy is still unclear, and some of the active ingredients have poor solubility, poor stability, low bioavailability and other problems after extraction. Therefore, future research requires more emphasis on the pharmacokinetic study of drug efficacy substances in the body, to clarify its mechanism of action in the human body. In addition, the research can also optimize the structure of the pharmacodynamic substances, so that they can be better used in the preparation of medicines, which is conducive to the synthesis and processing of their active ingredients by the pharmaceutical industry, thus enhancing the medications' effectiveness and improving their ability to prevent and treat influenza.

5.4. Lack of comparative experiments between Chinese and Western medicines

Current studies on the anti-influenza viruses of TCMs primarily focus on their own mechanism of action, and there is a lack of comparative efficacy analyses with Western medicines for the same influenza virus model. In the future, researchers can do more comparative experiments, so as to facilitate the clinical work to choose more efficient and more appropriate medicines to intervene the influenza virus.

5.5. Lack of research on influenza virus B

There are two types of viruses that have a significant impact on public health, namely, influenza virus A and B. However, most studies are currently focused on influenza virus A with a lack of research on influenza virus B, which could be supplemented by additional research.

6. Conclusion

This article finds that at this stage, researchers have attempted to study the mechanism of action of TCMs against influenza viruses from multiple angles, which can be broadly categorized into direct inhibition and indirect inhibition. Direct inhibition includes the inhibition of influenza virus surface protein HA, the inhibition of influenza virus surface protein NA and the inhibition of influenza virus nucleocapsid contents, and other mechanisms that directly affect the virus itself, including the inhibition of influenza virus autophagy and apoptosis, the inhibition of influenza virus M1 protein expression, and the inhibition of influenza virus replication enzymes, etc. Indirect inhibition is mainly through the enhancement of the body's immunity to fight against viral infection. Research has shown that the pathological damage

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caused by influenza infection is more likely to come from the excessive inflammatory response produced by the body, i.e. the "cytokine storm". It has been found that many TCMs can also reduce the damage caused by viral infection by suppressing the excessive immune response of the body.

The results of the study have certain suggestive effects on the choice of TCMs used by clinicians at different stages of the disease, which greatly improves the accuracy of the use of Chinese medicines in the clinic. Meanwhile, existing challenges such as unclear active ingredients, unclear effective dosage, lack of clinical trials, comparative experiments between Chinese and Western medicines, and research on influenza virus B, etc. It is expected that future researchers can pay more attention to the above issues.

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