



REVIEW ARTICLE

Current and Future Antiviral Medications to Treat Influenza: Mechanisms of Action

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DOI: 10.18297/jri/vol3/iss1/9

Received Date: February 1, 2019

Accepted Date: March 13, 2019

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Introduction

Due to significant research efforts in the field of influenza therapy, in the near future we will have a series of new antivirals to treat patients with influenza. Most likely, hospitalized patients with influenza pneumonia as well as other severe forms of influenza will be managed with combination antiviral therapy. During the selection of antivirals, it will be important for physicians to have a clear understanding of the mechanisms of action of anti-influenza medications.

In this manuscript I will review the main steps of the life cycle of the influenza virus, classify current and future antivirals according to the step that they inhibit in the viral life cycle, and summarize antivirals mechanisms of actions.

Life cycle of influenza virus

The influenza viruses reach to the human respiratory tract via droplets from a patient with active disease. Once in the respiratory tract, viruses infect and replicate in epithelial cells. The steps in the life cycle of influenza in a human epithelial cell are depicted in **Figure 1** [1-3]. The influenza virus contains three membrane proteins, hemagglutinin, neuraminidase, and matrix 2 (M2) protein. Hemagglutinin is the most abundant of the three viral proteins, followed by neuraminidase and M2. There are eight copies of viral RNA gene segments, viral RNA polymerase, and other core proteins inside of the virus (**Figure 1: Influenza virus**).

Step 1. Attachment of influenza virus to epithelial cell

The virus uses the surface glycoprotein hemagglutinin to attach to molecules of sialic acids present in the surface of epithelia

cells (**Figure 1: Attachment**). Since sialic acids are abundant in membranes of human epithelial cells, the virus has multiple receptors to attach. The binding of the hemagglutinin to sialic acid triggers the next step of viral entry into the cell.

Step 2. Endocytosis of the influenza virus

The virus enters the epithelial cell in an endosome (**Figure 1: Endocytosis**). Inside the endosome, due to the low pH, there is a conformational change of the viral hemagglutinin.

Step 3. Fusion of viral and endosomal membranes

The viral hemagglutinin fuses with the endosomal membrane (**Figure 1: Fusion**). At this point, the viral membrane is in contact with the endosomal membrane.


Step 4. Release of viral components into the cytosol

The low pH in the endosome is responsible for changes occurring in the M2 viral membrane protein, with opening of the M2 ion channels. As a consequence, the viral core is acidified and the viral components are released into the cell's cytoplasm (**Figure 1: Release**). From the cytoplasm, the viral proteins travel to the cell's nucleus.

Step 5. Transcription and replication of viral RNA

Once in the nucleus, the viral RNA polymerase carries out the transcription and replication of the influenza genome (**Figure 1: Replication of viral RNA**). Replication of the viral genome does not require a primer.

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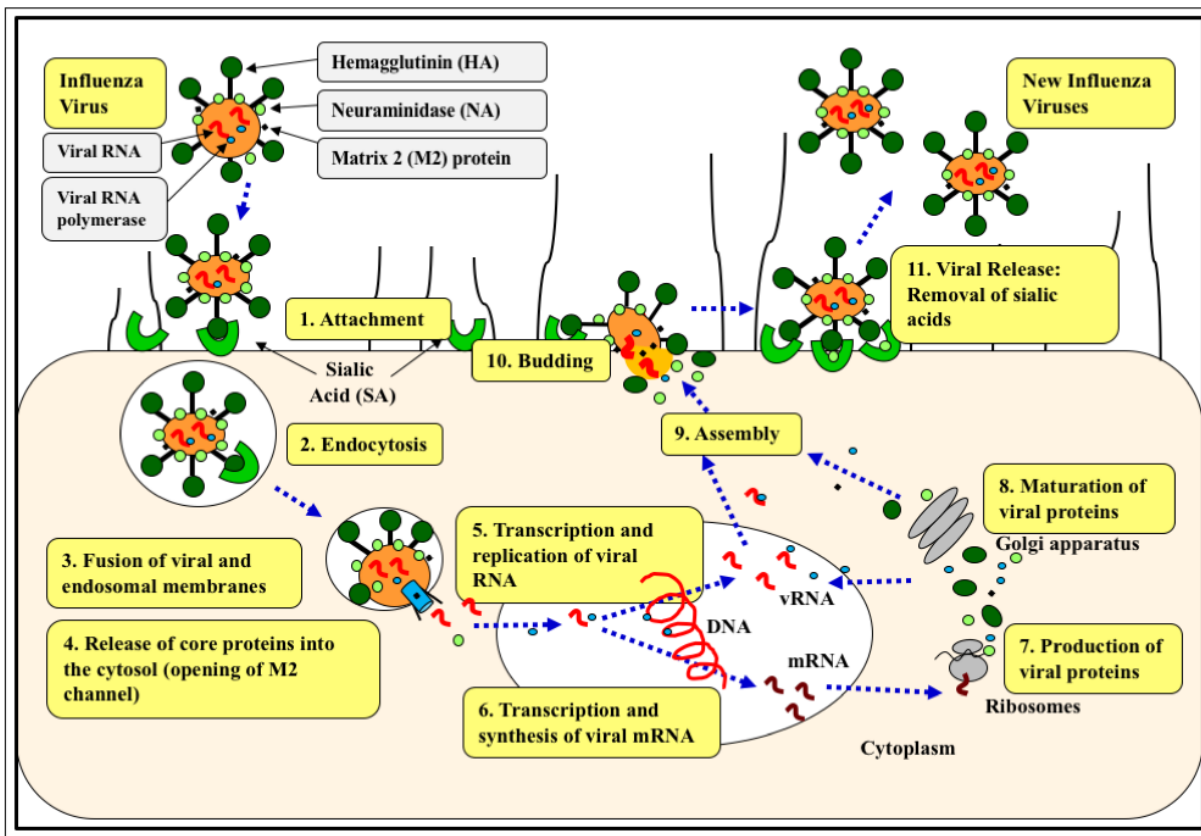


Figure 1 Steps of the influenza life cycle in a human epithelial cell E

Step 6. Transcription and synthesis of viral messenger RNA (mRNA)

The virus uses the viral RNA polymerase to generate mRNA (**Figure 1: Synthesis of viral mRNA**). The viral RNA polymerase is composed of three protein subunits, the PB2, PB1, and PA. During the process of generating viral mRNA, the viral RNA polymerase obtains a cap from the cellular mRNA, a process called “cap-snatching”. This generates short-capped oligomers that will serve as primers for transcription of viral mRNA. Once the viral mRNA is formed, it is moved from the nucleus to the cytoplasm.

Step 7. Production of viral proteins

The viral mRNA uses the cell’s ribosomes to translate all viral proteins (**Figure 1: Production of viral proteins**). Some proteins will return to the nucleus to form a complex with the newly formed viral RNA.

Step 8. Maturation of viral proteins

Several viral proteins are taken by the Golgi apparatus. As the proteins move through the apparatus they mature and become ready for assembly (**Figure 1: Maturation**).

Step 9. Assembly

The viral RNA and the new viral proteins accumulate in the cytoplasm, and a new virus will be assembled (**Figure 1: Assembly**). The nascent virus will bulge in the cell membrane.

Step 10. Budding

During the budding process, the virus will use some of the cell’s membrane to form the new viral envelope (**Figure 1: Budding**). The ion channel M2 protein contributes to the budding of the virus by functioning as a membrane-bending protein.

Step 11. Release of new viruses

Once the new influenza virus buds from the cell, the virus remains attached to the cell’s sialic acid receptor. The viral neuraminidase has sialidase activity. By altering sialic acid, the neuraminidase is able to release new viruses (**Figure 1: Viral release**).

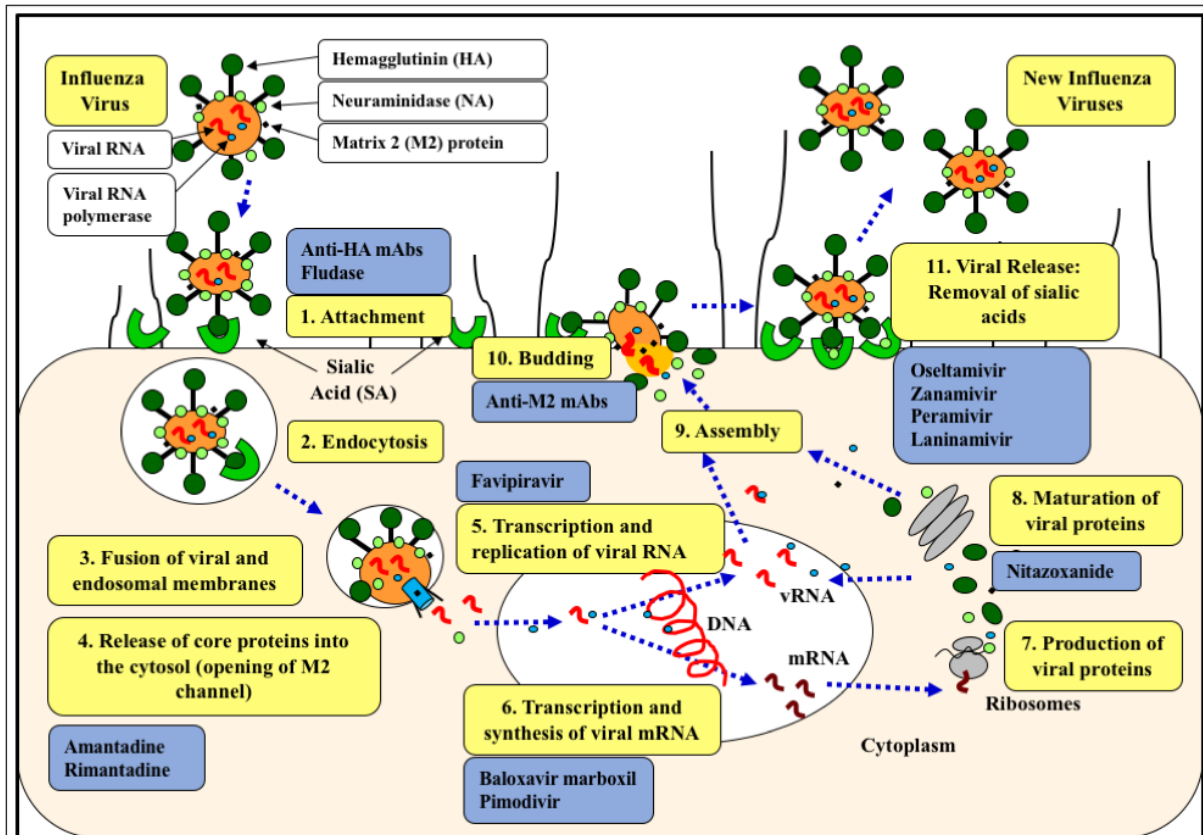


Figure 2 Classification of antivirals according to the step they inhibit in the influenza life cycle.

Antiviral medications to treat influenza

Antiviral medications classified according to the steps that they block in the viral life cycle are depicted in **Figure 2**. The mechanisms of action of each antiviral medication and spectrum of action against influenza A and B viruses will be reviewed.

Antivirals acting on Step 1: Attachment of influenza virus to epithelial cell

Anti-Hemagglutinin Monoclonal Antibodies

Several monoclonal antibodies are being developed which target the highly conserved stem region of the hemagglutinin molecule of the virus [4]. By blocking the hemagglutinin, the virus is not able to attach to the sialic acid receptors. These antibodies bind to the more conserved stalk portion of the hemagglutinin molecule, and thus, they have broad antiviral activity against several influenza A subtypes. Some of the antibodies in clinical development include MEDI8852, MHAA4549A, VIS- 410, CT-P27, CR6261, and CR8020. There is also in development an antibody targeting the hemagglutinin of influenza B (MHAB5553A).

Fludase (DAS181)

Fludase adheres to the membrane of the epithelial cells and cleaves the sialic acid from the cell surface [5]. Without sialic acid in the cell membrane, the virus has no receptor to attach. Fludase is delivered as an aerosolized nebulizer formulation, which allows the drug to remove the sialic acid of the epithelial cells in the area of infection. Since sialic acid is the human receptor for all influenza viruses, the drug has activity against all types of influenza viruses. This drug is not approved in the US.

Antivirals acting on Step 4: Release of viral components into the cytosol

Amantadine and Rimantadine

These drugs block the M2 ion channel protein, inhibiting the release of core viral proteins into the cytoplasm. They only block M2 ion channel proteins of influenza A viruses. Therefore, these medications are not active against influenza B viruses. Due to the high levels of resistance to these drugs among the currently circulating influenza A viruses, the CDC recommends that amantadine and rimantadine not be used in clinical practice [6].

Antivirals acting on Step 5. Transcription and replication of viral RNA

Favipiravir

Favipiravir is a potent inhibitor of the viral RNA polymerase and block the replication of the influenza viral genome [7]. The drug is ribosylated and phosphorylated intracellularly to form favipiravir-RTP, which is the active form of the drug. This form of the drug is recognized by the viral RNA polymerase and incorporated into an emerging viral RNA as a purine which results in chain termination during RNA synthesis. Favipiravir has a broad spectrum of activity against influenza viruses. The spectrum of activity also includes RNA viruses other than influenza. Favipiravir has a risk for teratogenicity and embryotoxicity. The drug is currently approved for influenza infection in Japan but is not approved in the US.

Antivirals acting on Step 6: Transcription and synthesis of viral mRNA

Baloxavir marboxil

Baloxavir targets the cap-dependent endonuclease in the PA subunit of the viral RNA polymerase and acts by inhibiting the process known as cap snatching [8]. This will block the production of the primers necessary for transcription of the viral mRNA. Baloxavir has activity against Influenza A and B viruses. The prodrug baloxavir marboxil is converted by hydrolysis to the active form, baloxavir acid. The drug, that is administered as a single dose by mouth, was recently approved in the US.

Pimodivir

Pimodivir targets the PB2 subunit of the viral RNA polymerase and acts by preventing the cap-snatching activity of the influenza viral polymerase [9]. Pimodivir inhibits the synthesis of viral mRNA of influenza A viruses, but is inactive against influenza B virus due to structural differences in the PB2 cap-binding pocket. This drug is not approved in the US.

Antivirals acting on Step 8: Maturation of viral proteins

Nitazoxanide

Nitazoxanide blocks maturation of the viral glycoprotein hemagglutinin [10]. The drug had no effect on the other glycoprotein, neuraminidase, or the M2 protein. The drug is orally administered and is deacetylated in the blood to the active metabolic form tizoxanide. The drug is not approved in the US for treatment of influenza.

Antivirals acting on Step 10: Budding of new viruses

Anti-M2 Protein Monoclonal Antibodies

There is an antibody targeting the M2 protein of the influenza A virus (TCN032) [11]. The antibody binds to the M2 which is exposed during the budding of the new virus on the surface of infected cells. The drug prevents virus budding and thus acts by a different mechanism from the M2 ion channel inhibitors. This drug is not approved in the US.

Antivirals acting on Step 11: Release of new viruses

Oseltamivir, Zanamivir, Peramivir, and Laninamivir

These drugs target the glycoprotein neuraminidase. By inhibiting the neuraminidase, they block the detachment of the virus from the sialic acid moieties on the epithelial cell. Neuraminidase inhibitors have activity against both influenza A and B viruses. Oseltamivir, zanamivir, and peramivir are approved in the US. Peramivir is an intravenous formulation [12]. Laninamivir is approved in Japan but not in the US [13].

Conclusions

Our options to treat hospitalized patients with influenza infection will continue to expand. Some of the new drugs have improved effectiveness compared to currently available drugs when treatment is initiated beyond 48 hours after symptom onset. Antiviral combinations with drugs which inhibit different steps of the influenza life cycle could become the standard approach for managing complicated patients. Combination therapy may also decrease the risk for the selection of viral resistance. Comprehensive understanding of the mechanisms of actions of

antiviral medications will be necessary when selecting therapy for patients hospitalized with severe influenza.

Funding Source: No funding sources declared.

Conflict of Interest: None reported.

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