 doi:10.20944/preprints202208.0008.v1

Review

Understanding Anti-Influenza Antiviral Drugs

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Abstract: Antigenic drift in influenza strains allows viruses to avoid being fully suppressed by seasonal vaccines. As a result, public interest has led to increased scrutiny and reevaluation of anti-influenza antiviral drugs as possible solutions. Unfortunately, many anti-influenza drugs developed around the globe suffer from a lack of sufficient clinical trials, as well as a lack of toxicity data. This is especially true of Arbidol, a popularly used drug for the prevention and treatment of influenza strains in China and Russia. Neuraminidase inhibitors, which were developed in the United States, also fall victim to inconclusive clinical trials and adverse effects. Adamantanes, while proven to be effective in treating influenza A, are encountering rapid, widespread cross-resistance. Baloxavir marboxil, a newer anti-influenza medication, shows promise in treating acute uncomplicated influenza, and may avoid the development of resistance when coadministered with other antiviral drugs. This review explores the antivirals available for influenzas treatment at this time.

Keywords: influenza; antivirals; anti-influenza; influenza drugs; the flu

1. Background

According to the CDC, influenza viruses are divided into four types: A, B, C, and D. The A and B strains infect human hosts and are the focus of this review. Type C influenza viruses are thought by the CDC to only cause mild respiratory distress, and not epidemics, while type D influenza viruses are only known to cause illness in cattle. Type A viruses can be further broken down into subtypes based on the gene composition of their surface proteins: the current subtypes of the A virus are H3N2 and the 2009 version of H1N1. The previous H1N1 strain was replaced by a different variant that emerged in 2009, which resulted in a pandemic (Influenza (Flu) CDC, 2022). Type B influenza viruses, which only infect humans, are not subtyped, but can be of either B/Yamagata or B/Victoria lineage (Influenza (Flu) CDC, 2022).

As seen in **Figure 1**, influenza viruses have two glycoproteins on their surfaces: hemagglutinin and neuraminidase. The presence of these two glycoproteins gives influenza viruses their ability to adapt to and evade host immune responses, which necessitates the invention of new preventative vaccines each flu season. Hemagglutinin is a sialic acid receptor–binding molecule that mediates entry of the influenza virus into a target cell and is therefore the main target for a host body's neutralizing enzymes (Koel et al., 2015). Neuraminidase enzymes are then responsible for cleaving the glycosidic linkages of viral neuraminic acids, which allows the entry of these new influenza particles to spread throughout the target cell (Moscona, 2005). A viral life cycle is composed of 5 stages; viral entry, viral uncoating, viral replication, assembly and budding, and viral release from the host cell (Yang, 2019). Each of these life stages, as well as their unique surface proteins, provide anti-influenza drugs ample opportunities for viral-specific modes of action.

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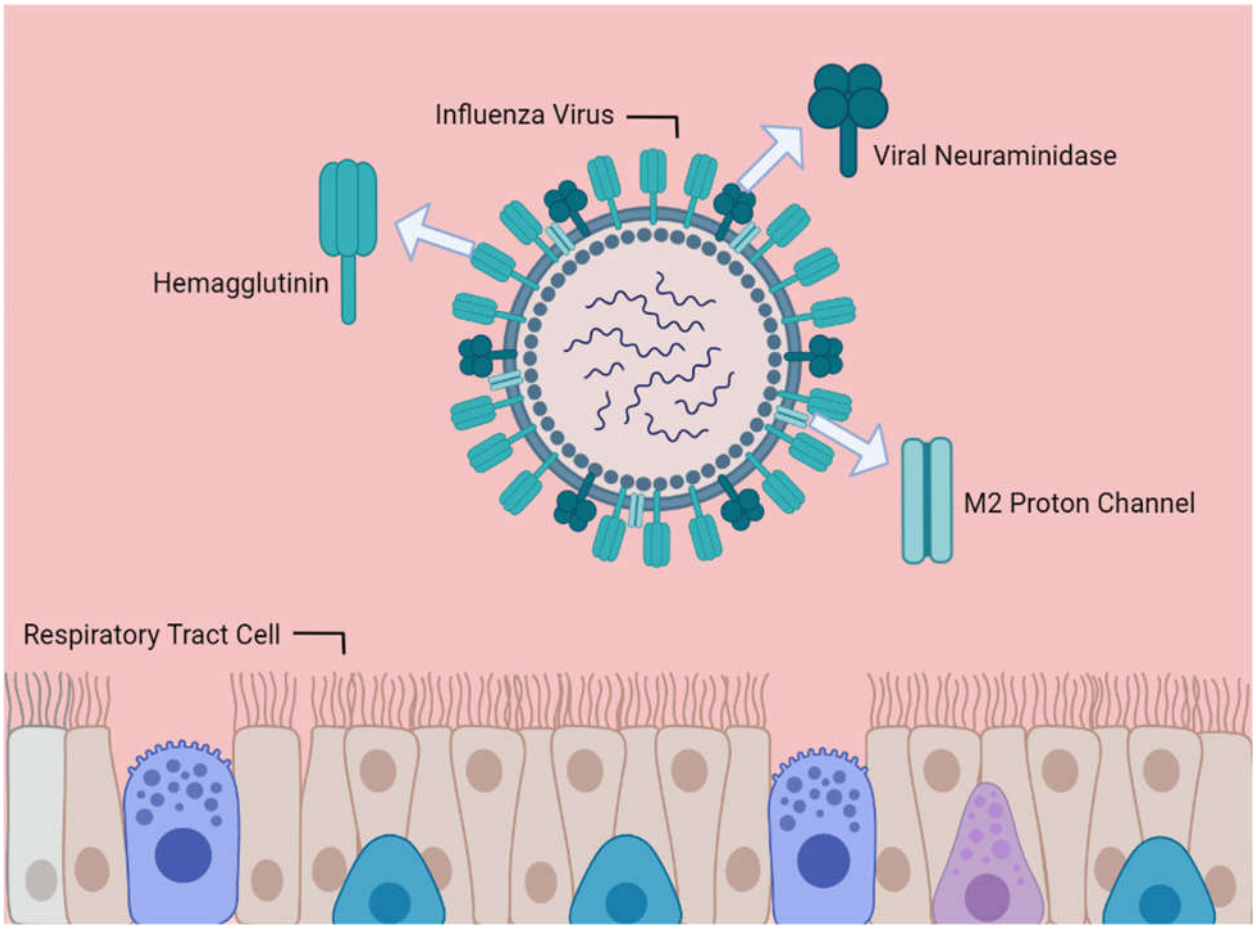

Figure 1. Structure of an Influenza Virus. The prominent viral coat structures are emphasized, including the two glycoproteins hemagglutinin and neuraminidase. The M2 proton channel is also displayed. Near the bottom of the figure is the surface of a respiratory tract. Made with BioRender.com.

As the COVID-19 pandemic has shown, viral evolution is becoming increasingly problematic. Even the most minute changes in viral code can result in lessened efficacy of vaccines and drugs; even rendering them impotent. Understanding the mechanisms by which viruses gain resistance and avoid vaccines is vital to developing an effective antiviral drug. There are two mechanisms in which influenza viruses genetically modify themselves to avoid antibodies and vaccines: antigenic drift and antigenic shift. In both antigenic drift and shift, hemagglutinin is the determining factor. Antigenic shift occurs when hemagglutinin in zoonotic influenza viruses adapt to a new host species through a function of its receptor binding (Koel et al., 2015). For antigenic drift, the viral evolution process accumulates mutations within the antibody binding sites of the hemagglutinin, and there is increasing concern over these mutations resulting in a drastic antigenic drift among the entire viral gene pool (Retamal et al., 2018). These mutations arise from amino acid substitution within the hemagglutinin protein and have yet to produce a mass viral antigenic drift thus far; despite seven years of constant evolution resulting in the 2016 H1N1 strains showing roughly 13 amino acid differences when compared with the 2009 H1N1 strain, there have been no changes in hemagglutinin activity or inhibition yet (Retamal et al., 2018). However, it has been noted that the reason the 2009 H1N1 strain was so potent was due to its amino acid changes at position 151 and 159, which are adjacent to its binding site and lead to its evasion from human antibodies. This type of evasion results in the reinfection of individuals with immunity to the previous strains, and thwarts seasonal vaccines (Koel et al., 2015). Regardless of the lack of incidence thus far, the possibility of severe antigenic drift happening in the near future is likely enough that preventative measures are currently being taken.

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As immunity to current influenza strains increases, the potential for mutant variants increases as well. There have been many studies performed globally to see if lab-created mutations of influenza viruses have the capacity to increase binding affinity, leading towards antigenic drift. One such study was performed in the Department of Viroscience in Rotterdam, The Netherlands, in conjunction with the University of Cambridge. This particular study, which began in response to the 2009 H1N1 influenza variant and was completed in 2015, focused on amino acid substitutions that would induce or support antigenic changes in the 2009 H1N1 strain. Certain amino acid substitutions within the hemagglutinin protein could result in the influenza virus escaping neutralizing antibodies, increased viral fitness, and changes in receptor preferences (Koel et al., 2015). These changes can lead to the sudden inefficiency of not only the current antiviral drugs on the market, but the influenza vaccines as well, which would lead to increased influenza mortality. In this study, researchers took the 2009 H1N1 Netherlands strain and constructed amino acid substituted variants in order to explore the molecular changes that contribute to antigenic drift. The researchers found that four of their single acid substitutions caused substantial antigenic drift (Koel et al., 2015). Notably, this high mutation rate, along with the fact that single substitutions caused substantial antigenic change, contradicts the low rate at which influenza viruses have changed antigenically naturally. One explanation for this is that substitutions responsible for escape from antibodies have an adverse effect on hemagglutinin function, which slows down the emergence of new antigenic variants (Koel et al., 2015).

A follow up study was performed at the Research Center in Infectious Diseases of the CHU of Quebec and Laval University, centered on the potential of the 2009 H1N1 strain of influenza to achieve antigenic drift perpetuated by escape mutations in the hemagglutinin sialic acid antigenic binding site (Retamal et al., 2017). The researchers rescued single and combined hemagglutinin mutant viruses in the H1N1 viral backbone, and six of their 11 amino acid substitutions found in the escape mutants were located in the predicted antigenic sites. However, the remaining five mutations were located outside the reported antigenic sites. These two sets were then introduced into the California H1N1 2009 strain, and the most antigenically altered virus was tested for its ability to overcome the current vaccine in infected mice (Retamal et al., 2017). Their results indicted a likely occurrence of drift variants caused by G158E and N159D substitutions within the sialic acid antigenic region of the HA1 subunit in the near future (Retamal et al., 2017).

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2. Anti-influenza Drugs

While there are many anti-influenza drugs currently in the developmental pipeline, very few have been approved for use in humans. Most anti-influenza drugs currently available have limited toxicity data, and in some cases the risk of poorly understood side effects outweigh the meager therapeutic benefits gained from taking the drug. For the treatment, prevention, and management of post influenza complications, there are a handful of drug classes to choose from.

2.1. Umifenovir

Umifenovir, also known as Arbidol, is a broad-spectrum antiviral that acts against viral hemagglutinin specifically (Borskin et al, 2008). Umifenovir is currently only approved in Russia and China for treatment of influenza A and B, prophylaxis, and post influenza complications (Haviernik et al., 2018). It is also a controversial drug; due to its questionable effectiveness and limited toxicity data, it has yet to be approved for use in the United States.

Umifenovir is thought to be an inhibitor of various enveloped and non-enveloped RNA viruses, including influenza strains (Xiong et al., 2007). The suspected mode of action of umifenovir is based on its insertion into membrane lipids, leading to the inhibition of membrane fusion between virus particles and plasma membranes, as well as interfering with the fusion between virus particles and the membranes of endosomes (**Figure 2**). In influenza strains, umifenovir interacts with hemagglutinin, causing an increase in hemagglutinin stability and prevents its transition into its fusing state. Umifenovir may also be immunomodulatory, which would allow it to interfere with induction and macrophage activation (Haviernik et al., 2018). Proper dosage of umifenovir is variable, but one study showed that at 50 or 100 mg/kg/day, 24 hours before virus exposure, for 6 days, umifenovir significantly reduced the rate of infections and rate of mortality in mice infected with an influenza A strain (Xiong et al., 2007).

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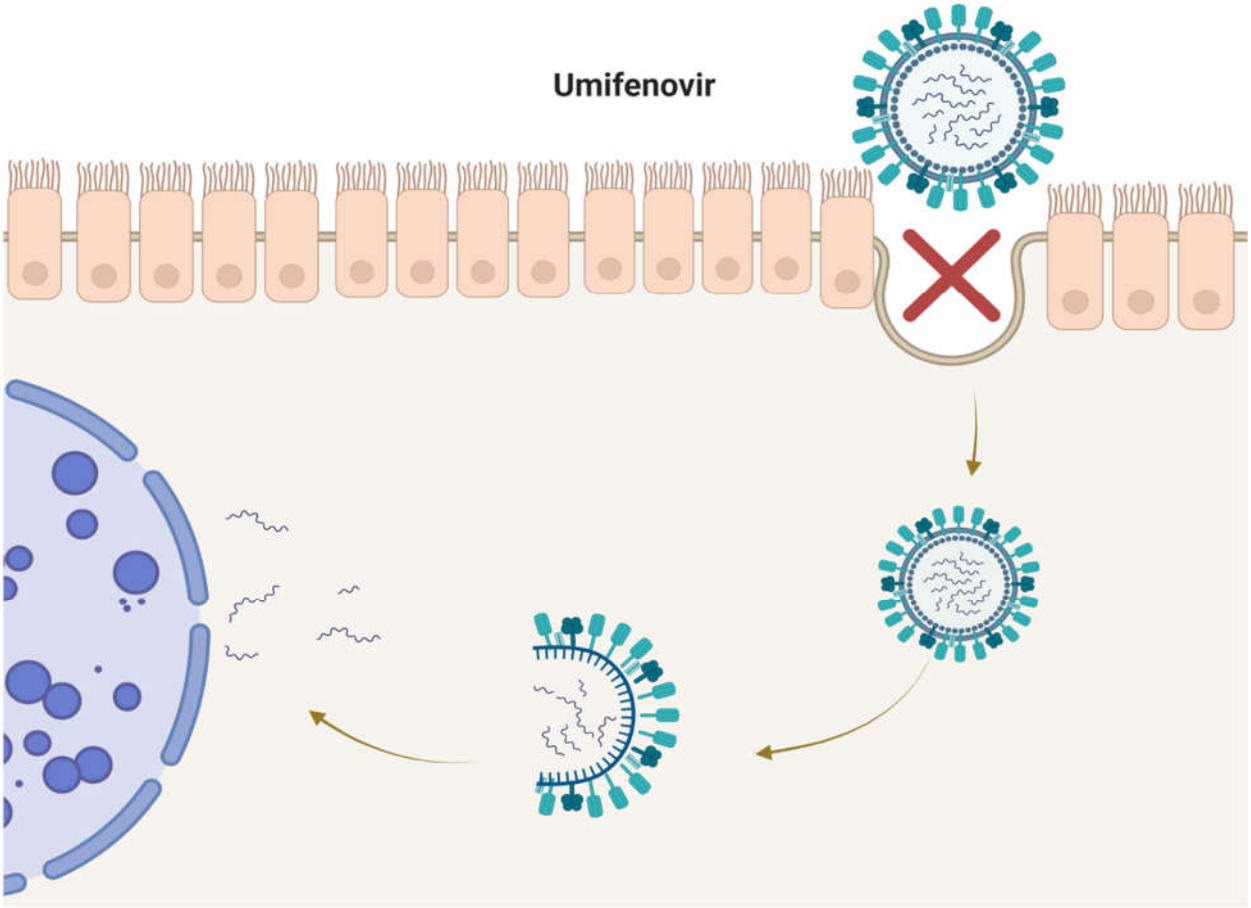

Figure 2. Proposed umifenovir mechanism. Current understanding of umifenovir's method of action is based on its insertion into membrane lipids, leading to the inhibition of membrane fusion between virus particles and plasma membranes, as well as interfering with the fusion between virus particles and the membranes of endosomes. Made with BioRender.com.

A study out of Russia recently tested the effectiveness of umifenovir on post-influenza complications, specifically S. aureus pneumonia following infection of the California 2009 H1N1 strain in mice. This study proposed that oral 40 or 60 mg/kg/day doses increased the survival rate in mice from 0% to 90%. The researchers also claim that the treatment decreased the amount of virus present in the lungs of the mice, as well as the viable bacteria counts. After dissection, they also found that the lungs of treated mice had less severe histopathologic lesions when compared to the control group (Leneva et al., 2014).

The many studies conducted out of Russia and China indicate that umifenovir is an effective, broad-spectrum antiviral that works against a number of human pathogenic respiratory viruses, but its actual effectiveness is still in question in many other countries due to a lack of reproducible lab results (Xiong et al., 2007). The FDA has yet to approve umifenovir in the United States, but its mass use in China and Russia makes it worth mentioning.

2.2. Neuraminidase Inhibitors

Neuraminidase inhibitors are a class of drugs that inhibit the actions of neuraminidase enzymes. Of the four approved antiviral agents for the treatment of influenza approved in the United States at the time of writing, three of them, oseltamivir (Tamiflu), zanamivir (Relenza Diskhaler), and peramivir (Rapivab) are neuraminidase inhibitors. As seen in **Figure 3**, neuraminidase inhibitors target viral release (Yang, 2019). Neuraminidase cleaves the terminal sialic acid from the carbohydrate residue on the surface of the host cells, which the influenza virus envelopes. This promotes the release of the virus from the infected cells, which allows the virus to spread. Neuraminidase inhibitors block the

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active site of this enzyme, which reduces viral shedding (Yang, 2019) **Figure 3** shows how replication is blocked by neuraminidase inhibitors, which prevents virions from being released from the surface of infected host cells (Moscona, 2015).

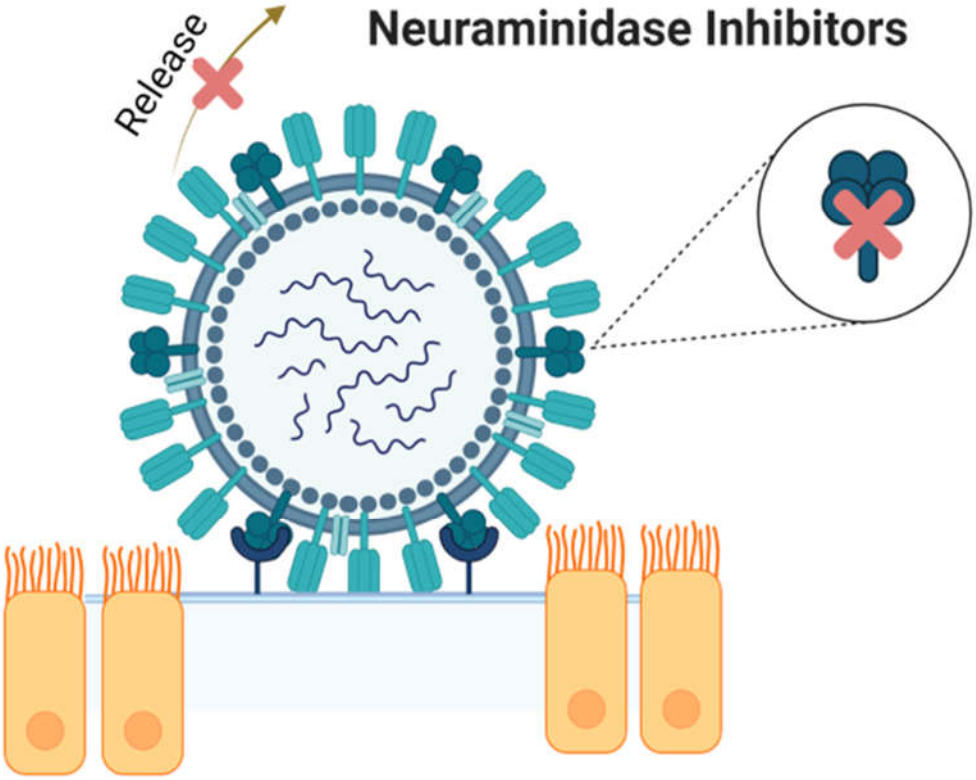

Figure 3. Neuraminidase inhibitors. These antivirals prevent neuraminidase from acting on terminal sialic acid from the carbohydrate residue on the surface of the host cells, thereby inhibiting viral release and further replication. Made with BioRender.com.

Whether or not oseltamivir is an effective treatment for Influenza A and B strains has been questioned recently, due to the sloppy clinical trials involving the drug. Many of the clinical trials contained bias, and several possibly had an active substance as their placebo. Several studies have concluded that neuraminidase inhibitors have small, unspecific effects that result in shortening the duration of influenza symptoms by about 24 hours, but not in all patients. While using neuraminidase inhibitors for prophylaxis has been shown to be effective, the use of oseltamivir increases the chance of adverse effects, such as nausea, vomiting, psychiatric effects, and renal events in adults, along with vomiting in children. Zanamivir produces less adverse effects than the other two drugs in this class, possibly due to its lower bioavailability, while peramivir produces the most adverse effects, possible due to its IV route of administration (Jefferson et al., 2014). The balance between their potential adverse effects and their potential benefits should be carefully weighed before administration. Regardless, the FDA has approved these three neuraminidase inhibitors for the prophylaxis and treatment for avian influenza strains in the United States, as well as for treatment for post-influenza effects.

2.3. M2 Inhibitors (Adamantanes)

Adamantanes are a class of anti-influenza drugs that were used specifically for treating type A influenza infections, but mass viral resistance has limited their recent use.

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There are only two members of this class; amantadine (Symmetrel) and rimantadine (Flumadine), which are both symmetric tricyclic amines. Adamantanes are also called M2 inhibitors, or M2 ion-channel inhibitors, based upon their mode of action. Amantadine and rimantadine specifically inhibit the replication of influenza A strains (Ison and Hayden, 2017). As seen in **Figure 4**, M2 ion-channel inhibitors target the stage of viral uncoating. The M2 proteins are responsible for forming the proton channels that lower the pH of the viral interior right before the dissociation of matrix protein, which eventually leads to the uncoating of the viral genome during replication (Yang, 2019).

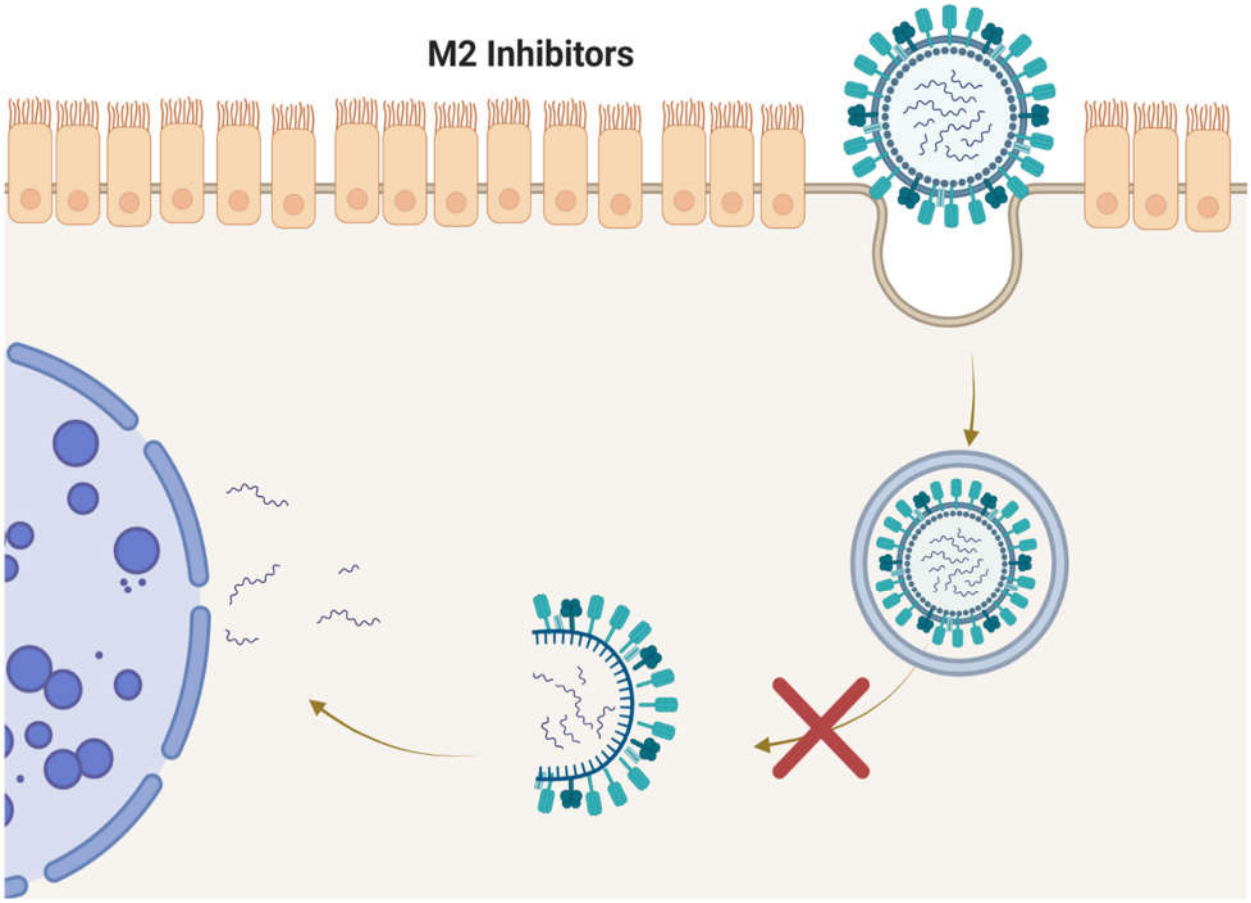

Figure 4. M2 Ion-channel Inhibitors. These antivirals target the stage of viral uncoating and prevent it from happening altogether. This halts the virus from proceeding to the replication stage. Made with BioRender.com.

Amantadine and rimantadine are given in similar oral dosage amounts; 100mg tablets and a syrup formulation of 50mg/5mL (Ison and Hayden, 2017). The dosage for adults, for treatment and prevention of influenza A, is 100mg every 12 hours. Both drugs achieve peak levels within the body at around 3-5 hours after dosing. Amantadine is excreted unchanged by the kidney, but rimantadine undergoes extensive hepatic metabolism before renal excretion. Common side effects of adamantanes are minor CNS complaints such as anxiety, difficulty concentrating, insomnia, dizziness, and headaches, as well as GI upset. Rarer, but well documented side effects include antimuscarinic effects, orthostatic hypotension, and congestive heart failure. Drug–drug interactions can occur with a large amount of drug classes, including antihistamines and anticholinergic drugs, which further limits their use (Ison and Hayden, 2017).

Rimantadine is structural analog of amantadine and is seen as the superior drug due to its larger volume of distribution, higher concentrations in respiratory secretions, and more extensive metabolism that results in fewer CNS side effects. However, rimantadine shares its specificity, mechanism of action, and potential for resistant with amantadine

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(Mondal, 2016). Cross-resistance to both drugs occurs when a single amino acid is substituted in the transmembrane portion of the M2 protein. Resistance has been seen to emerge as soon as 2–4 days after the start of therapy, in up to 30% of patients infected with strains that showed susceptibility to either drug. Because of the widespread resistance to M2 inhibitors in influenza A strains, these drugs are not currently recommended for the prevention or treatment of influenza in the United States (Ison and Hayden, 2017).

2.4. Cap-Dependent Endonuclease Inhibitors

Cap-dependent endonuclease (CEN) is present in the RNA polymerase subunit in influenza viruses. Cap-dependent endonuclease mediates the cap-snatching process of viral mRNA biosynthesis, which is vital for viral reproduction (Portsmouth et al., 2017). Baloxavir Marboxil (baloxavir) is a newly FDA-approved member of the antiviral class of cap-dependent endonuclease inhibitors (Yang, 2019). Baloxavir is a prodrug that is metabolized via hydrolysis into its active metabolite, baloxavir acid. Baloxavir acid targets the replication stage of the viral life cycle, and selectively inhibits the endonuclease activity of the polymerase acidic protein, one of the subunits of RNA polymerase. The targeted endonuclease is a virus-specific enzyme required for viral gene transcription, which provides baloxavir its specificity. Through inhibition of cap-dependent endonuclease, baloxavir is able to inhibit influenza viral replication for both influenza A and B viruses (Yang, 2019) (**Figure 5**).

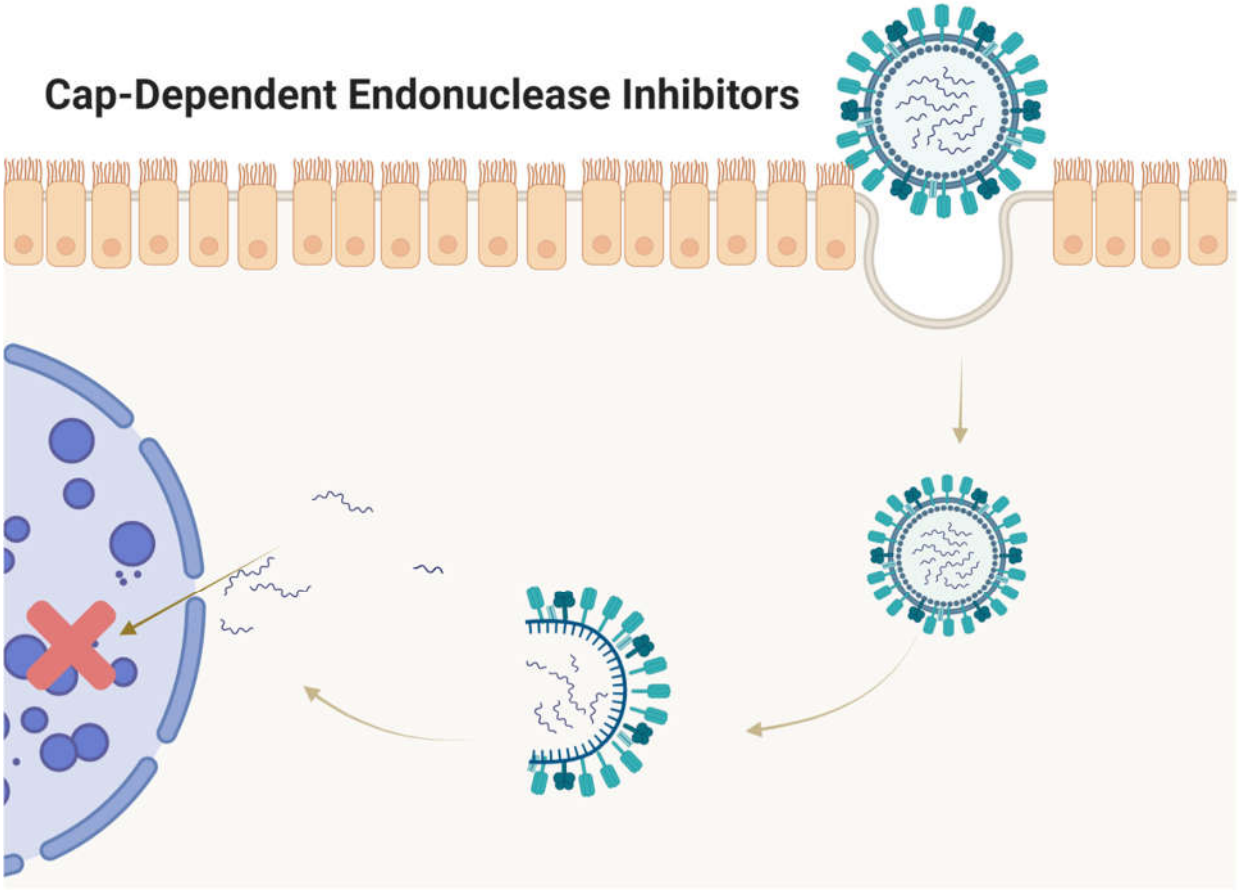

Figure 5. Cap-dependent Endonuclease Inhibitors. These antivirals target the replication stage of the viral life cycle, and selectively inhibit the endonuclease activity of the polymerase acidic protein, one of the subunits of RNA polymerase. Through inhibition of cap-dependent endonuclease, these antivirals inhibit influenza viral replication. Made with BioRender.com.

Baloxavir is indicated for use in patients 12 years and older who have been symptomatic for a maximum of 48 hours, and only for acute uncomplicated influenza. In practice,

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this makes baloxavir an inferior alternative to oseltamivir and zanamivir, which are also indicated for prophylaxis and can be used for patients that are 2 weeks old, and 7 years old, respectively. Baloxavir is the preferred choice in patients where use of neuraminidase inhibitors is contraindicated. Baloxavir has a half-life of 79.1 hours; because of this, it is given in a single-dose regimen (Yang, 2019).

Baloxavir is metabolized in the liver mainly by the enzyme UGT1A3, with minor contributions by CYP3A4. To date, no serious drug-drug interactions have been documented, even with coadministered CYP3A and UGT inhibitors such as probenecid. Coadministration with medicines containing polyvalent cations, such as antacids, lowers the bioavailability of baloxavir. Baloxavir is mainly excreted in the feces, with minor excretions in the urine, and in patients with renal and hepatic impairments, baloxavir showed no altered pharmacokinetic properties (Yang, 2019).

While baloxavir can treat viruses resistant to oseltamivir, the main problem in using baloxavir is the speed by which viruses develop resistance towards it. In a recent study, it was found that viruses would substitute at I38 in the polymerase acidic protein, which resulted in reduced susceptibility to baloxavir (Yang, 2019). However, when coadministered with oseltamivir, synergistic properties were shown between the two drugs, and resistance was avoided (Fukao et al., 2018). This suggests that baloxavir may be most effective when included in a treatment regimen that includes other antiviral drugs.

3. Conclusion

Antigenic drift in influenza strains allow these viruses to circumvent seasonal vaccines. Because of this, recent public interest, as well as recent scientific interest, has led to the reevaluation of older anti-influenza drugs, as well as the development of new anti-influenza drugs. Unfortunately, because they are still a relatively new type of antiviral, anti-influenza drugs suffer from a lack of efficacy data. Anti-influenza drugs also lack a wealth of toxicity data, especially for drugs developed in Russia and China. As each class of antiviral possesses a unique mode of action, the variety of anti-influenza drugs available could help prevent resistance from cropping up quickly among the various influenza strains, if used in combination with each other. Ultimately, new antivirals, novel combinations of current antivirals, and coadministration with other preventative measures such as vaccines are the best ways to combat seasonal antigenic drift.

Acknowledgments: Special thanks to the van Staaden lab at Bowling Green State University for the use of BioRender, as well as to Dr. Steinmiller at The University of Toledo for proofreading. This review fulfills an MSPS requirement for the author.

Conflicts of Interest: The author declares that the literature search was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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