Review

Popular Influenza Antiviral Drugs: Mechanisms, Efficacy, and Resistance

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Abstract: Influenza viruses cause acute respiratory infections responsible for significant mortality and morbidity around the world. Because factors such as antigenic drift allow influenza strains to avoid being fully suppressed by seasonal vaccines, public interest has led to increased scrutiny of antivirals as treatment and prophylaxis options for seasonal outbreaks and potential pandemics. Unfortunately, many influenza antivirals suffer from a lack of sufficient clinical trials, as well as a lack of toxicity data; this is especially true of umifenovir (Arbidol), a popularly used drug for the prevention and treatment of influenza strains in China and Russia. Neuraminidase inhibitors, though widely prescribed, display a potential for future resistance. Adamantanes, while proven to be effective in treating influenza A, are already encountering rapid, widespread cross-resistance, and are effectively obsolete. Baloxavir marboxil, a newer antiviral, shows promise in treating acute uncomplicated influenza and may avoid the development of resistance when coadministered with other antiviral drugs. Indeed, the low genetic barriers to resistance faced by influenza antivirals may be surmounted by coadministration with other antivirals. This review explores the most widely prescribed antivirals for influenza treatment, their mechanisms of action, and current data on their susceptibility to resistance and efficacy at this time.

Keywords: influenza; antivirals; umifenovir; neuraminidase inhibitors; adamantanes; cap-dependent endonuclease inhibitors

1. Introduction

Influenza viruses reside in the *Orthomyxoviridae* family. Three of these viruses, influenza A, B, and C, are known to infect humans and cause acute respiratory infections [1]. Influenza A is prone to antigenic variation and is capable of interspecies transmission; this variant is often the cause of major flu pandemics [2-4]. Influenza viruses have the glycoproteins hemagglutinin (HA) and neuraminidase (NA) on their surface, as well as Matrix-2 (M2) proton channels (**Figure 1**). The presence of HA and NA gives influenza viruses their ability to adapt to and evade host immune responses, which necessitates the invention of new preventative vaccines each flu season.

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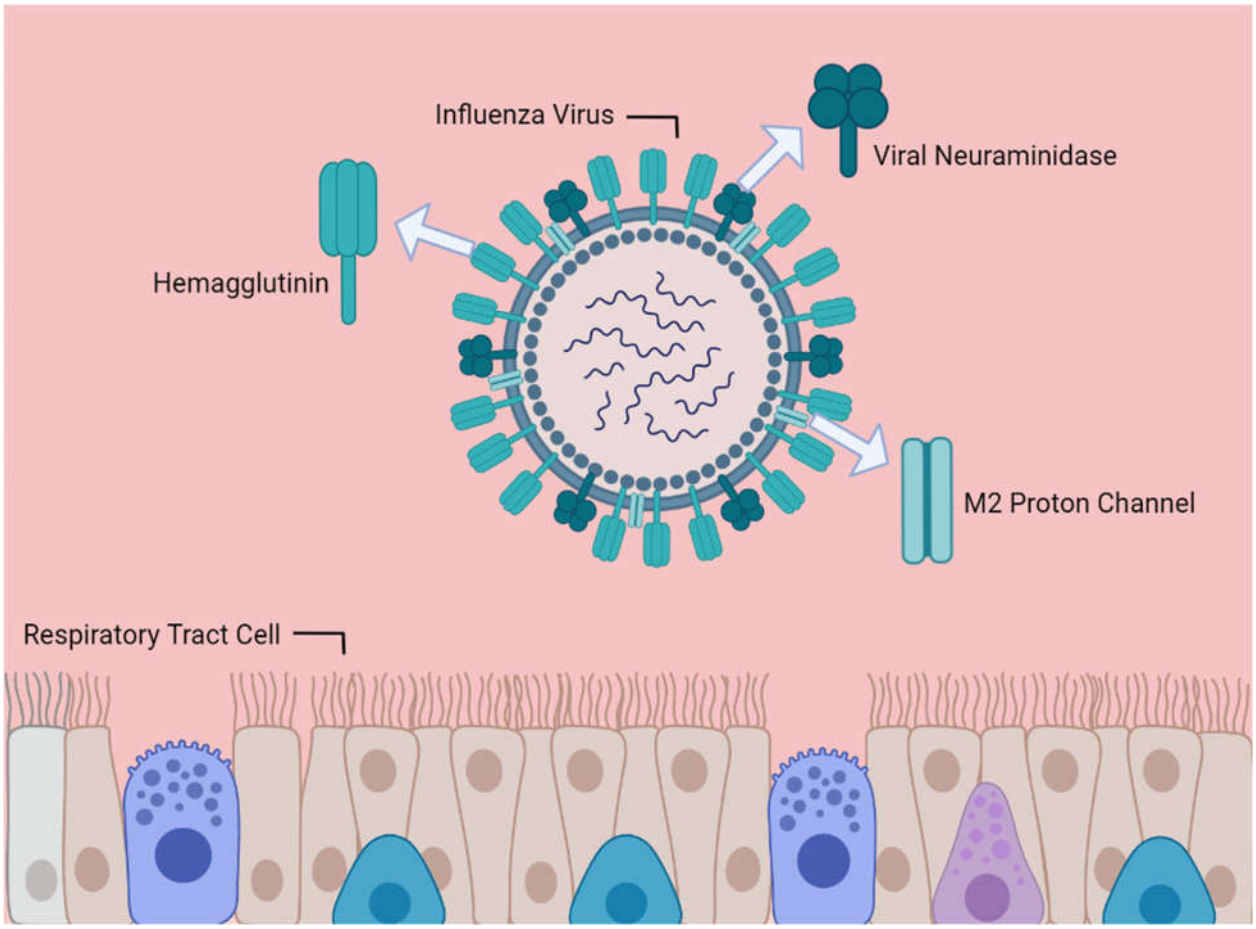

Figure 1. Structure of an influenza virus. The prominent viral coat structures are emphasized, including the two glycoproteins hemagglutinin and neuraminidase. The M2 proton channel is also displayed. Near the bottom of the figure is the surface of a respiratory tract. Made with BioRender.com.

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 [5]. HA 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 [6]. NA 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 [7]. These unique surface proteins, as well as each viral life stage, provide influenza antivirals different targets for therapeutic action.

While there are various influenza antiviral drugs currently in the developmental pipeline, very few have been approved for use in humans. Currently, for the treatment, prevention, and management of post-influenza complications, there are a handful of drug classes to choose from. This review will explore the uses, mechanisms, emerging resistance, and current efficacy data on the most widely prescribed antivirals, including umifenovir, the three most widely used NA inhibitors (oseltamivir, zanamivir, and peramivir), the M2 inhibitors, and the cap-dependent endonuclease inhibitor baloxavir marboxil. Other influenza antiviral drugs exist but are not as widely prescribed, such as laninamivir and favipiravir, which are approved for influenza treatment in Japan as of 2010 [8] and 2014 [9], respectively. These, and others like them, have limited efficacy data and clinical studies, and lack information on the potential for viral resistance, all of which currently prevents their widespread use. As such, they, and others like them, will not be talked about here.

2. Umifenovir

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Umifenovir (Arbidol) is a broad-spectrum antiviral that acts against viral HA specifically [10]. Developed in the 1970s by the collaborative efforts of the Chemical–Pharmaceutical Scientific Research Institute of Russia, the Scientific Research Institute of Medical Radiology in Obninsk, and the Leningrad-Pasteur Scientific Research Institute for Epidemiology and Microbiology, Umifenovir is currently only approved in Russia and China for treatment of influenza A and B, prophylaxis, and post-influenza complications [11-13], though it does exhibit anti-influenza C activity as well [10]. Umifenovir is a controversial drug; due to a lack of reproducible lab results [14] and limited toxicity data outside of Russia, it has yet to gain global use, and remains unapproved for influenza treatment in many countries. Information on umifenovir is difficult to find in the west, largely due to the language barrier, as key information, such as early clinical trial designs and results, is often only available in Russian [13]. There are however many Russian reports describing umifenovir's anti-influenza activity against strains such as influenza A(H5N1) and the 2009 A(H1N1) variant [15-17].

Umifenovir is thought to be an inhibitor of various enveloped and non-enveloped RNA viruses, including influenza strains [10, 18]. 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**) [10, 14, 18]. In influenza strains, umifenovir interacts with HA, causing an increase in HA stability and preventing its transition into the fusing state [19-21]. Umifenovir may also be immunomodulatory, which would allow it to interfere with induction and macrophage activation [11]. Umifenovir is also proposed to have antioxidant activity, which is thought to counteract virus activity [22]. As this drug is not well known outside of Russia and China, this section will examine recent and notable *in vitro*, *in vivo*, and clinical studies pertaining to umifenovir's efficacy as an influenza treatment.

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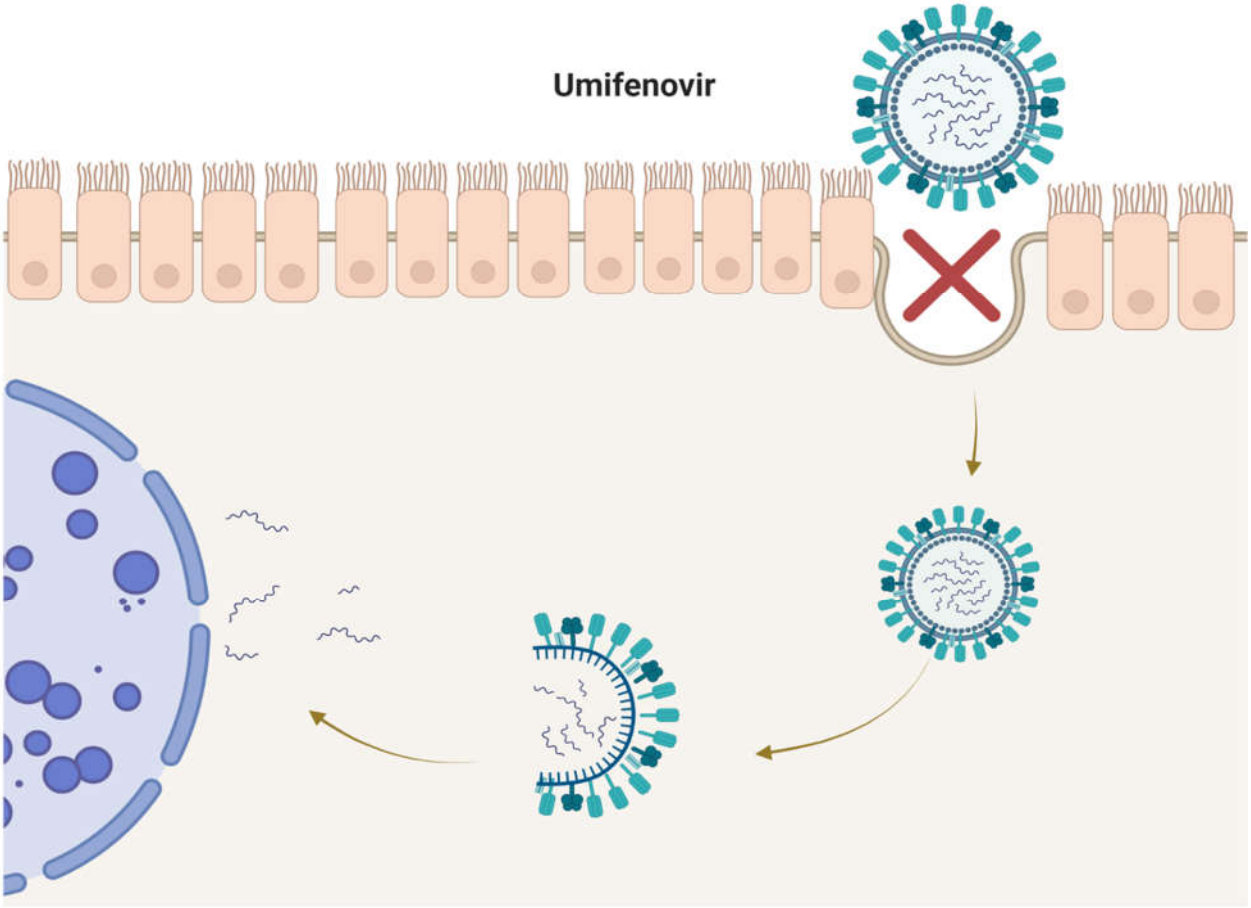

Figure 2. Proposed umifenovir mechanism. Current understanding of umifenovir's method of action is based on its insertion into membrane lipids, leading to the inhibition of membrane fusion between virus particles and plasma membranes, as well as interfering with the fusion between virus particles and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

Russian *in vitro* studies are plentiful and report IC50s for umifenovir in the 2.5–16 μM range [13, 15, 16, 23-25]. One of the best sources of information on this drug currently comes from the I.I. Mechnikov Research Institute of Vaccines and Sera, Russian Academy of Medical Sciences, Moscow, Russia, and its affiliates. Most notably, these labs have performed tests *in vivo* [25, 26], *in vitro* [17, 21, 23, 27-29], and clinical trials [30, 31] on the effectiveness of umifenovir against influenza strains, as well as other types of viruses. A recent *in vitro* study from this group showed, using a MDCK cell-based enzyme-linked immunosorbent assay, that influenza A and B viruses from the 2012-2014 flu seasons were inhibited by umifenovir, and no markers of resistance were found in viruses isolated from umifenovir-treated patients [25]. Their second *in vitro* study examined nasal swabs from 57 umifenovir-treated patients, with influenza A(H1N1), A(H3N2) or influenza B strains, and found no sign of resistance [26]. An *in vivo* study from this group also showed that umifenovir was effective against influenza A(H3N2) in orally treated mice at daily doses of 15 mg/kg or 20 mg/kg [30]. Another notable *in vivo* study explored the effectiveness of umifenovir on post-influenza complications, specifically *Staphylococcus aureus pneumonia* following infection of the California 2009 A(H1N1) strain in mice. This study showed that oral 40 or 60 mg/kg/day doses increased the survival rate in mice from 0% to 90%, and after dissection the lungs of treated mice displayed less severe histopathologic lesions when compared to the control group [26].

Two clinical studies from this group also examined patients with either influenza or acute respiratory tract infection. The first clinical trial enrolled 215 patients aged 18-74 years and split them into placebo (n=106) and treatment (n=109) groups. The treatment group received umifenovir 200 mg four times a day for 5 days [31]. The second clinical

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trial enrolled 359 patients aged 18-65 years and split them into treatment (n=181) and placebo (n=178) groups. The treatment group received 800 mg/day for 5 days [30]. For both trials, both the influenza and acute respiratory tract infection patients were grouped together; though patients in the umifenovir treatment group in both trials recovered faster overall and displayed less complications, it is difficult to parse out what the results mean for umifenovir's efficacy against influenza alone [30]. These studies reported no adverse effects attributed to umifenovir.

Umifenovir efficacy testing has been performed by labs in other countries as well, though studies are once again scarce. Studies out of China report the efficacy of umifenovir against influenza A variants; an *in vivo* study from Wuhan University showed that at doses of 50 or 100 mg/kg/day, 24 hours before virus exposure, for 6 days, umifenovir significantly reduced the rate of infections and mortality in mice infected with an influenza A strain [18]. An *in vitro* study also conducted out of Wuhan University showed that umifenovir was effective against two influenza A(H1N1) strains responsible for seasonal and pandemic influenza, respectively, in MDCK cells via a MTT assay [32]. Afterwards this same group conducted an *in vivo* study on mice and found that umifenovir treatment at oral doses of 90-180 mg/kg/day reduces viral lung titers and lesions. Additionally, the secretion of lung and macrophage cytokines were downregulated [32]. A more recent *in vitro* study from the First Affiliated Hospital of Guangzhou Medical University showed that umifenovir inhibited other local influenza A(H1N1) variants, including A(H3N2) and A(H9N2), with IC50s ranging from 4.4 to 12.1μM [33]. Their *in vitro* experiment performed shortly after on mice and ferrets showed that the survival rates of influenza-infected mice given 25 mg/mL and 45 mg/mL umifenovir were 40% and 50%, respectively, and displayed reduced viral lung titers. Their ferret data also showed a decrease in fever symptom duration in the umifenovir treatment groups compared to controls [33]. A clinical trial conducted by the Department of Respiratory Disease, Beijing, China, tested the efficacy of umifenovir on influenza on 125 influenza infected patients; 59 were in the treatment group and 66 were in the placebo group. This clinical study reports that at a dose of 200 mg orally 3 times per day for 5 days, the treatment group saw a significant reduction in symptoms and a median duration of illness of around 72 hours, compared to the placebo group's 96 hours. Adverse effects were not attributed to umifenovir [34].

From the Department of Biotechnology and Environmental Biology, RMIT University, Bundoora, Victoria, Australia, *in vivo* and *in vitro* testing revealed that while umifenovir neither reduced lung viral titers nor caused a significant reduction of lung consolidation in mice after oral, intraperitoneal administration, and intranasal challenge with a local influenza A(H3N2) strain, in cells, the therapeutic indices for influenza A and B were 1.9-8.5, and umifenovir was more effective against influenza A(H3N2) than rimantadine or amantadine [14]. Overall, the available studies indicate that umifenovir is an effective, broad-spectrum antiviral that works against a number of human pathogenic respiratory viruses, but its actual effectiveness will remain in question until lab results are globally reproducible.

3. Neuraminidase Inhibitors

NA inhibitors target viral release specifically and are effective against influenza A and B [35]. NA inhibitors, as their name suggests, are a class of drugs that inhibit the actions of NA enzymes [35]. NA 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 [35]. NA inhibitors block the active site of this enzyme, which reduces viral shedding [5, 35]. In this way, replication can be blocked by NA inhibitors, which prevents virions from being released from the surface of infected host cells (**Figure 3**) [7].

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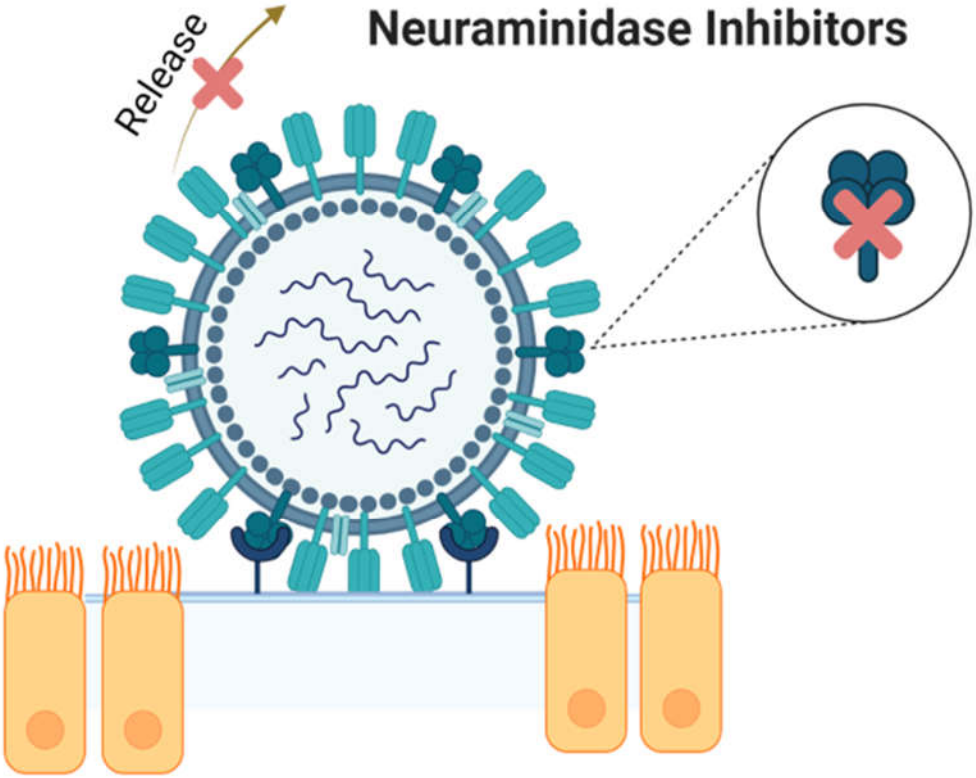

Figure 3. Neuraminidase inhibitors. These antivirals prevent neuraminidase from acting on terminal sialic acid from the carbohydrate residue on the surface of the host cells, thereby inhibiting viral release and further replication. Made with BioRender.com.

As of the time of writing, of the four approved antivirals for the treatment of influenza approved in the United States, three of them, oseltamivir (Tamiflu), zanamivir (Relenza Diskhaler), and peramivir (Rapivab), are NA inhibitors [36]. The recommended oseltamivir dosage for the treatment of acute influenza infection in adults, beginning within 2 days of symptom onset, is 75 mg orally twice daily for 5 days [37]. For prophylaxis, oseltamivir can be taken once daily for up to 42 days [38, 39]. Oseltamivir is taken as a prodrug (oseltamivir phosphate) and converted by hepatic esterases into its active metabolite oseltamivir carboxylate, which has a high bioavailability [38]. The recommended zanamivir dosage for the treatment of acute influenza in adults, beginning within 2 days after symptom onset, is 10 mg via oral inhalation twice daily for 5 days [40]. For prophylaxis, zanamivir can be taken once daily for up to 28 days [40]. Up to 15% of the dose is absorbed in the lungs [7, 40]. The recommended dosage of peramivir for the treatment of acute influenza in adults, beginning within 2 days after symptom onset, is a single dose of 600 mg intravenously [41]. Peramivir displays a low binding affinity to human plasma (<30%) [41], but in healthy adult volunteers, the peak concentration of peramivir in both pharyngeal and bronchial epithelial lining fluid samples was greater than the IC50 value for influenza [42].

Whether or not NA inhibitors are truly effective treatments for influenza A and B has been questioned in the past due to the sloppy clinical trials involving the drugs [43]. One large meta-analysis found that many of the clinical trials contained bias, and several possibly had an active substance as their placebo [43]. Several studies concluded that NA inhibitors shorten the duration of influenza symptoms, but not in all patients [43-49]. While using NA inhibitors for prophylaxis has been shown to be effective as well, the use of oseltamivir increases the chance of adverse effects, such as nausea, vomiting,

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psychiatric effects, and renal events in adults, along with vomiting in children [43]. Zanamivir produces less adverse effects than the other two drugs in this class, possibly due to its lower bioavailability and inhalation route, while peramivir produces the most adverse effects, possible due to its intravenous route of administration [43]. The balance between their potential adverse effects and their potential benefits should be carefully weighed before administration.

Resistance for NA inhibitors are drug-specific but given the similar structure shared by the drugs in this class, resistance to one can affect the activity of the others. Amino acid substitutions in either the NA catalytic site or the HA receptor binding site of influenza viruses can cause resistance to NA inhibiters to arise [50]. The H275Y amino acid substitution of the neuraminidase gene found in various influenza A viruses provides resistance towards oseltamivir and peramivir, E119E/V, found in influenza A(H3N2) and A(H7N9) provides resistance to oseltamivir, and R292K produces resistance to all three NA inhibitors, though lower resistance rates are observed for zanamivir [50-52]. While resistance to NA inhibitors can crop up in circulating strains, it is generally seen as rare [53-55], especially for zanamivir [56]. Regardless of its rarity, close monitoring for global NA inhibitor susceptibility is still ongoing [50].

4. M2 Inhibitors (Adamantanes)

Adamantanes are a class of anti-influenza antivirals that were used specifically for treating type A influenza infections, but mass viral resistance has limited their recent use. There are only two members of this class; amantadine hydrochloride (Symmetrel) and rimantadine hydrochloride (Flumadine), or simply amantadine and rimantadine, both of which are symmetric tricyclic amines [57]. Adamantanes are also called M2 inhibitors, or M2 ion-channel inhibitors, based upon their mechanism of action [58]. 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 the matrix protein, which eventually leads to the uncoating of the viral genome during replication [5, 59]. By inhibiting these ion channels, amantadine and rimantadine specifically inhibit the replication of influenza A strains (**Figure 4**) [60].

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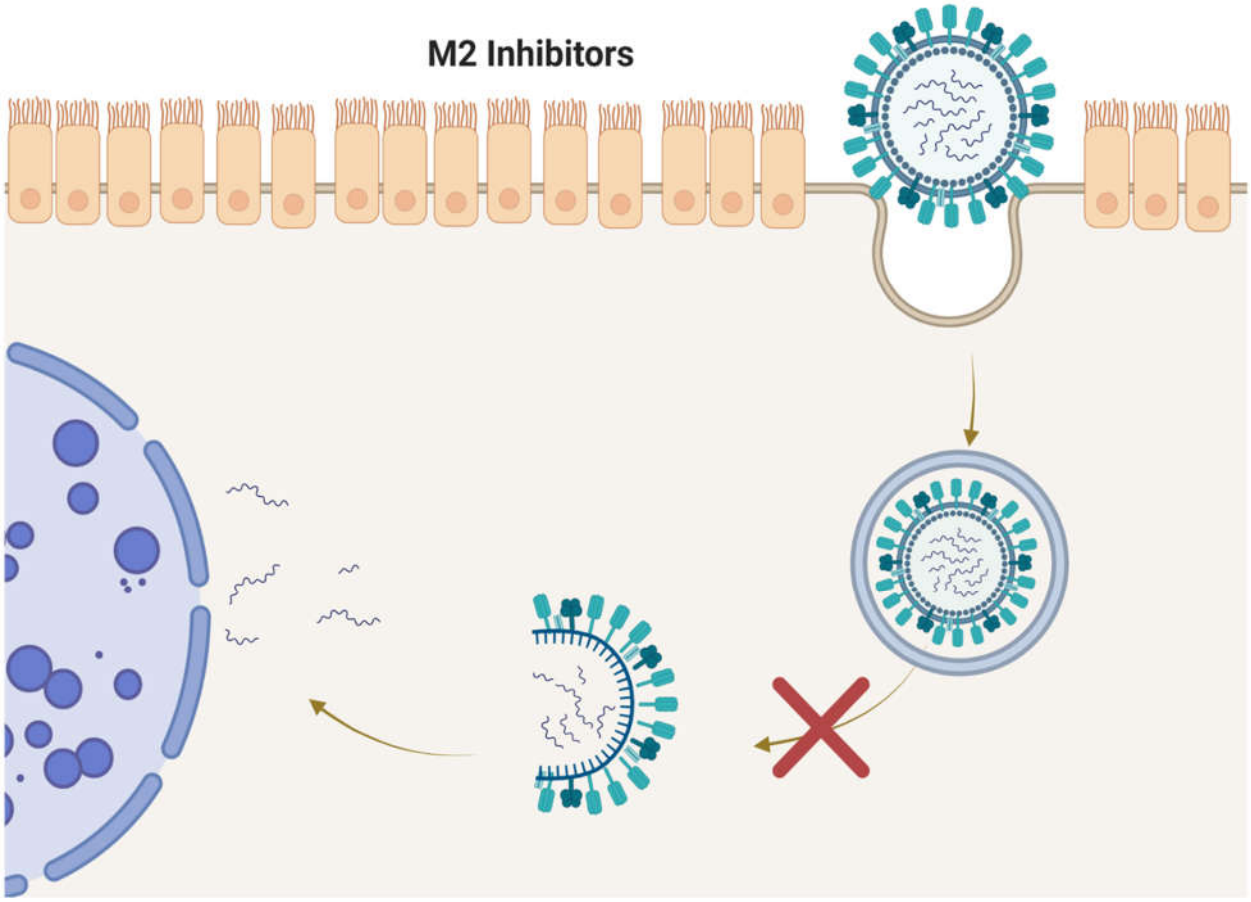

Figure 4. M2 ion-channel inhibitors. These antivirals target the stage of viral uncoating and prevent it from happening altogether. This stops the virus from proceeding to the replication stage. Made with BioRender.com.

Amantadine and rimantadine are given in similar oral dosage amounts; 100 mg tablets and a syrup formulation of 50 mg/5mL [60]. The dosage for adults, for the treatment and prevention of influenza A, is 100 mg every 12 hours. Both drugs achieve peak levels within the body at around 3-5 hours after dosing. Amantadine is excreted unchanged by the kidneys, but rimantadine undergoes extensive hepatic metabolism before renal excretion [61, 62]. Common side effects of adamantanes are minor central nervous system complaints such as anxiety, difficulty concentrating, insomnia, dizziness, and headaches, as well as gastrointestinal 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 [60, 63, 64].

Rimantadine is the 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 central nervous system side effects [60, 65]. However, rimantadine shares its specificity, mechanism of action, and potential for resistant with amantadine [66]. 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 [60]. Many studies throughout the years have shown influenza resistance to this drug class [67-75]. Because of the widespread resistance to M2 inhibitors exhibited by influenza A strains, these drugs are not currently recommended for the prevention or treatment of influenza in the United States [60, 72, 73].

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5. Cap-Dependent Endonuclease Inhibitors

Cap-dependent endonuclease is present in the RNA polymerase subunit in influenza viruses, where it mediates the cap-snatching process of viral mRNA biosynthesis, which is vital for viral reproduction [76]. Baloxavir marboxil (Xofluza), or baloxavir, was approved for the treatment of uncomplicated influenza first in Japan and the United States in 2018, followed shortly thereafter by a number of other countries [77, 78], making it the sole approved member of the antiviral class known as cap-dependent endonuclease inhibitors [5]. Baloxavir is a prodrug that is metabolized via hydrolysis into its active metabolite, baloxavir acid [79]. 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 [80]. The targeted endonuclease is a virus-specific enzyme required for viral gene transcription [81], which provides baloxavir its specificity. Through inhibition of cap-dependent endonuclease, baloxavir can inhibit influenza viral replication for both influenza A and B viruses [5, 79] (**Figure 5**).

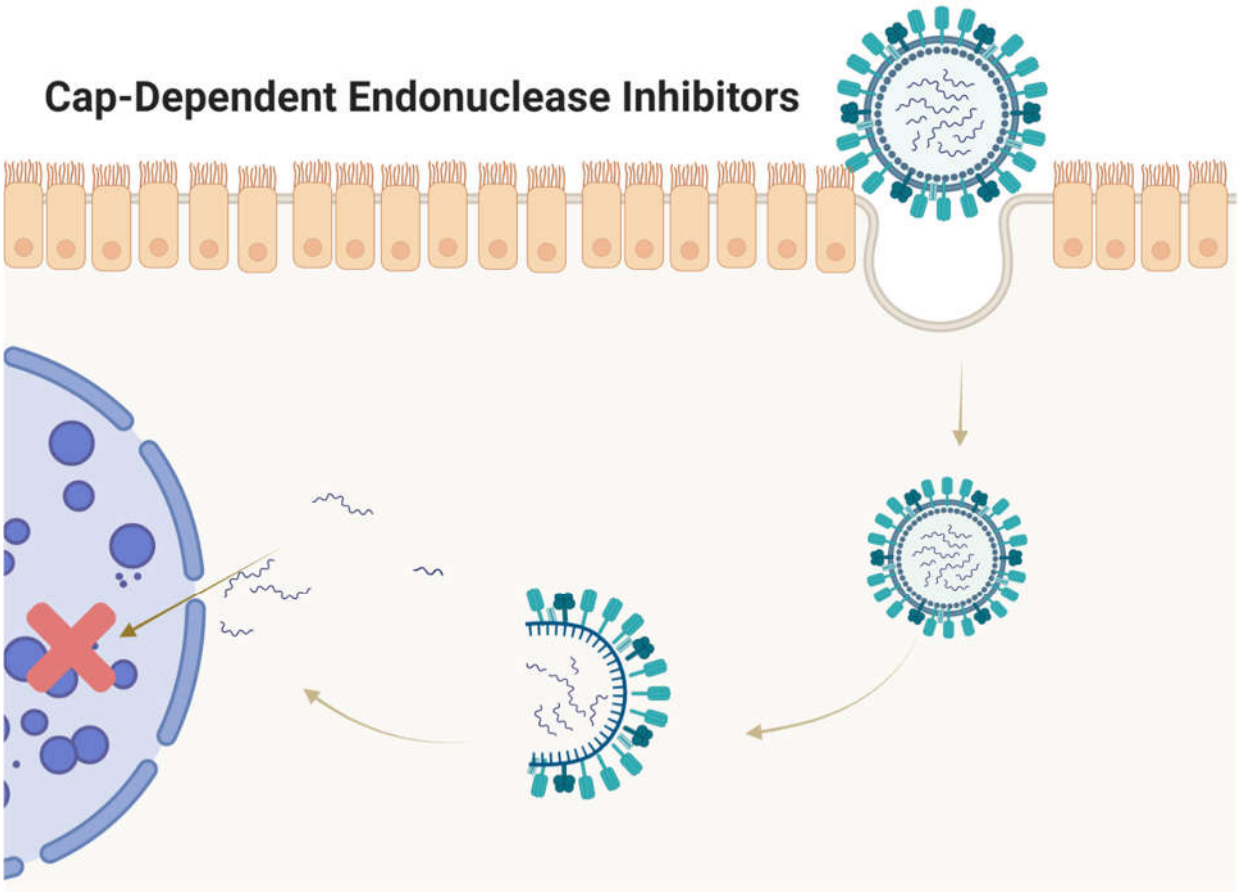

Figure 5. Cap-dependent endonuclease inhibitors. These antivirals target the replication stage of the viral life cycle, and selectively inhibit the endonuclease activity of the polymerase acidic protein, one of the subunits of RNA polymerase. Through inhibition of cap-dependent endonuclease, these antivirals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

Baloxavir is 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 [5, 82]. 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 [5, 82]. Baloxavir is indicated for use in patients 12 years and older who have been symptomatic for a maximum of 48 hours, and

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only for acute uncomplicated influenza [5, 82]. In this regard, baloxavir is an inferior alternative to other antivirals that are also generally indicated for prophylaxis as well as influenza treatment. Baloxavir however is the preferred choice in patients where the use of NA inhibitors is contraindicated, and because baloxavir has a half-life of about 79 hours, it is given in a single-dose regimen [5, 82]. In this regard, baloxavir is a superior treatment to other multi-dose regimens, as patient compliance is an issue with multiple dose treatment plans.

While baloxavir can treat viruses resistant to NA inhibitors, the main problem in using baloxavir alone is the speed by which influenza viruses develop resistance towards it. Both influenza A and B can develop resistance, though A more so than B [5]. In an *in vitro* study, it was found that viruses would substitute at I38 in the polymerase acidic protein, which resulted in reduced susceptibility to baloxavir [1]. Indeed, one clinical study that used baloxavir to treat mainly influenza A(H3N2) reported that even after a single dose of baloxavir, a small subset of influenza patients developed resistance to baloxavir, with a rate of 19.5% resistance overall [2]. Other clinical studies show resistance appearing at varying rates; 8%-10% has been reported when treating the same strain in a different study [3]. Interestingly, this study's previous results only reported a resistance rate of 2.2%, but the patients treated previously had contracted the 2009 A(H1N1) variant, the strain responsible for the 2009 pandemic [2].

In more recent years, baloxavir resistance was only observed at rates of 0.5% and 0.1% for the 2018-2019 and 2019-2020 flu seasons, respectively [4]. These results imply that baloxavir resistance varies across influenza strains, and remains a valid choice for treatment [5]. Additionally, when coadministered with oseltamivir, synergistic properties were shown between the two drugs, and resistance and drug-drug interactions were avoided [6-8]. A recent study showed a lack of drug-drug interactions between baloxavir NA inhibitors as well, though it failed to report improved clinical outcomes when compared to treatment plans consisting of a single antiviral [9]. These results suggest that, if widespread viral resistance to baloxavir, NA inhibitors, or both occur in the future, coadministering baloxavir with a NA inhibitor may be the most effective treatment regimen to bypass resistance.

6. 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 antivirals, as well as the development of new anti-influenza antivirals. Unfortunately, low genetic barriers to resistance will continue to be a problem for existing antivirals in the future. Even now, adamantanes are not recommended for widespread use due to the speed of resistance seen even after a single dose. Careful global monitoring of antiviral susceptibility to resistance is needed to ensure that one of the current few antivirals available for the treatment of influenza does not end up obsolete in the same manner. Considering the low genetic barriers to resistance when given individually, combination therapy utilizing two or more antivirals may be a way to circumvent viral resistance, at least in the short term. As each class of antiviral possesses a unique mechanism of action, the variety of anti-influenza antivirals available could help prevent resistance from cropping as up quickly among influenza strains. Ultimately, new antivirals, novel combinations of current antivirals, and coadministration with other preventative measures such as vaccines are the best ways to combat influenza.

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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.

Abbreviations: HA (hemagglutinin), NA (neuraminidase), M2 (Matrix-2)

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