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Popular Influenza Antiviral Drugs: Mechanisms, Efficacy, and Resistance

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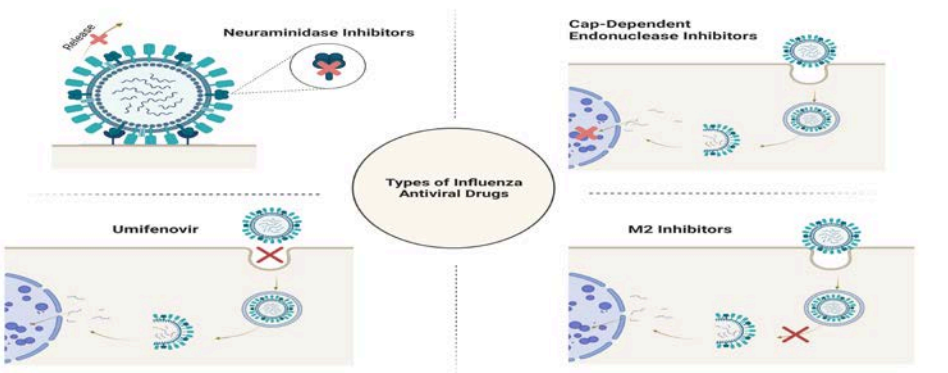
ABSTRACT
Influenza viruses cause acute respiratory infections responsible for significant mortality and morbidity around the world. Various factors, such as antigenic drift, allow influenza strains to avoid being fully suppressed by seasonal vaccines. This has led to the increased scrutiny of antivirals as treatment and prophylaxis options for seasonal outbreaks and potential pandemics. Unfortunately, many influenza antivirals suffer from a lack of adequate clinical trials, as well as a lack of toxicity data. This is especially true of umifenovir (arbidol), a drug popularly used 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 and 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 co-administered with other antiviral drugs. Indeed, the low genetic barrier to resistance associated with influenza antivirals could potentially be overcome by co-administration with other antivirals. This review explores the most widely prescribed antivirals for influenza treatment, their mechanisms of action, and the data currently available about their susceptibility to resistance and efficacy.
Keywords: adamantanes, antivirals, cap-dependent endonuclease inhibitors, influenza, neuraminidase inhibitors, umifenovir

GRAPHICAL ABSTRACT



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1. INTRODUCTION

Influenza viruses belong to the *Orthomyxoviridae* family. Among these, influenza A, B, and C, are known to infect human hosts and cause acute respiratory infections [1]. Influenza A is prone to antigenic variation and is capable of interspecies transmission. Moreover, 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.

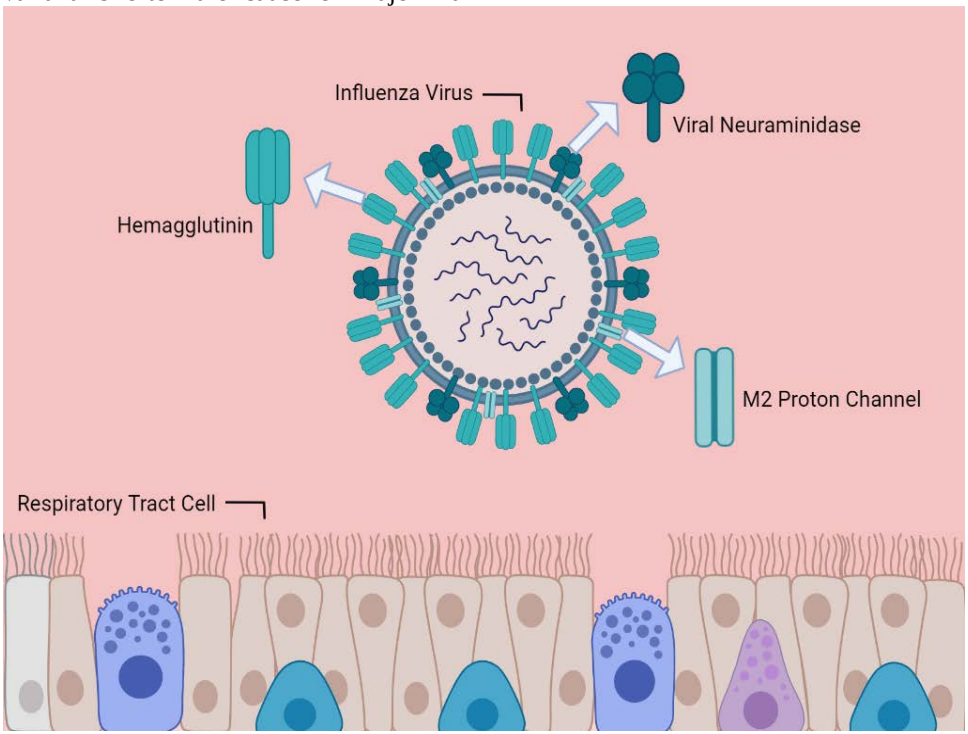

Figure 1. Structure of an Influenza Virus

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 the respiratory tract. Made with BioRender.com.

A viral life cycle is composed of five (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 the entry of the influenza virus into the 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 release of these new influenza particles to spread throughout the infected organism [7]. These unique surface

proteins, as well as each viral life stage, provide influenza antivirals with different targets for therapeutic action.

There are various influenza antiviral drugs currently in the developmental pipeline. However, very few have been approved for use by human patients. Currently, for the treatment, prevention, and management of post-influenza complications, there are a handful of drug classes to choose from. This review explores the uses, mechanisms, emerging resistance, and current efficacy data of 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. These include laninamivir and favipiravir, which were approved for influenza treatment in Japan in 2010 [8] and 2014 [9], respectively. These, and others like them, have limited efficacy data, and clinical studies lack information about the potential for viral resistance, which currently prevents their widespread use. As such, they, and others like them, are not discussed in this research.

2. UMIFENOVIR

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 approved only in Russia and China for the 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 including early clinical trial designs and results is often available only in Russian [13]. There are, however, many Russian reports describing umifenovir's anti-influenza activity against various strains, such as influenza A (H5N1) and the 2009 A (H1N1) variant [15–17].

Umifenovir is considered an inhibitor of various enveloped and non-enveloped RNA viruses 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 shows antioxidant activity, which presumably counteracts virus activity [22]. As this drug is not well known outside of Russia and China, this section examines the recent and notable *in vitro*, *in vivo*, and clinical studies about umifenovir's efficacy as influenza treatment.

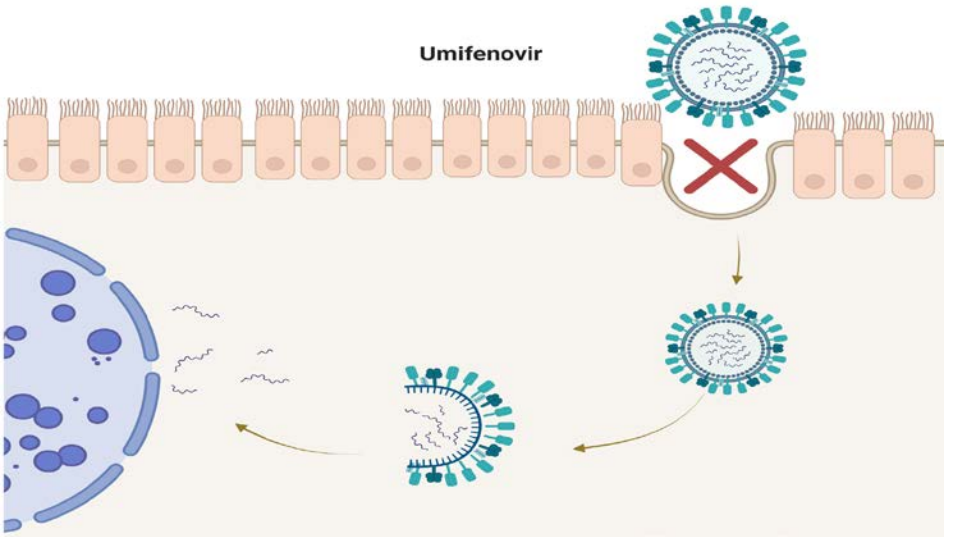

Figure 2. Proposed Umifenovir Mechanism

The 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 is 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] gauging the effectiveness of umifenovir against influenza strains, as well as other types of viruses. A recent *in vitro* study showed, using an MDCK cell-based enzyme-linked immunosorbent assay, that influenza A and B viruses from the 2012-2014 flu seasons were inhibited by umifenovir. Moreover, no markers of resistance were found in viruses isolated from umifenovir-treated patients [25]. Another *in vitro* study examined nasal swabs from 57 umifenovir-treated patients, with influenza A(H1N1), A(H3N2), and influenza B strains and found no sign of resistance [26]. An *in vivo* study also showed that umifenovir was effective against influenza A(H3N2) in orally treated mice at the daily doses of 15 mg/kg or 20 mg/kg [30]. Another notable *in vivo* study explored the effectiveness of umifenovir in post-influenza complications, specifically *Staphylococcus aureus pneumonia*, following the 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%. Furthermore, after dissection, the lungs of the treated mice displayed less severe histopathologic lesions as compared to the control group [26].

Two clinical studies 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 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]. In both trials, both the influenza and acute respiratory tract infection patients were grouped. The patients in the umifenovir treatment group in both trials recovered faster and displayed fewer complications. Still, 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 in labs in other countries as well, though such studies remain scarce. Studies out of China reported the efficacy of umifenovir against influenza A variants. An *in vivo* study from Wuhan University showed that 24 hours before virus exposure, at doses of 50 or 100 mg/kg/day for 6 days, umifenovir significantly reduced the rate of infection and mortality in mice infected with an influenza A strain [18]. An *in vitro* study also conducted at Wuhan University showed that umifenovir was effective against two influenza A(H1N1) strains, responsible for both seasonal and pandemic influenza, in MDCK cells via an MTT assay [32]. Afterward, an *in vivo* study on mice found that umifenovir treatment at oral doses of 90-180 mg/kg/day reduced viral lung titers and lesions. Additionally, the secretion of lung and macrophage cytokines was downregulated [32]. A more recent *in vitro* study from the First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China 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]. The *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. Moreover, these mice displayed reduced viral lung titers. The ferret data also showed a decrease in fever symptoms duration in umifenovir treatment groups as compared to controls [33]. A clinical trial conducted by the Department of Respiratory Diseases, in Beijing, China tested the efficacy of umifenovir on influenza on 125 influenza-infected patients. Of these patients, 59 were in the treatment group and 66 were in the placebo group. This clinical study reported that at a dose of 200 mg, administered 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].

At the Department of Biotechnology and Environmental Biology, RMIT University, Bundoora, Victoria, Australia, both *in vivo* and *in vitro* testing revealed that umifenovir neither reduced lung viral titers nor caused a significant reduction of lung consolidation in mice after oral and intraperitoneal administration and intranasal challenge with a local influenza A(H3N2) strain. In cells, the therapeutic indices for influenza A and B were in the range of 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 and broad-spectrum antiviral that works against

several human pathogenic respiratory viruses, although its actual effectiveness remains in question until lab results are reproducible, globally.

3. NEURAMINIDASE INHIBITORS

NA inhibitors target the viral enzyme neuraminidase to inhibit viral release 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 host cells, which influenza virus envelopes. This promotes the release of the virus from the infected cells which, in turn, 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 prevent virions from being released from the surface of the infected host cells (Figure 3) [7].

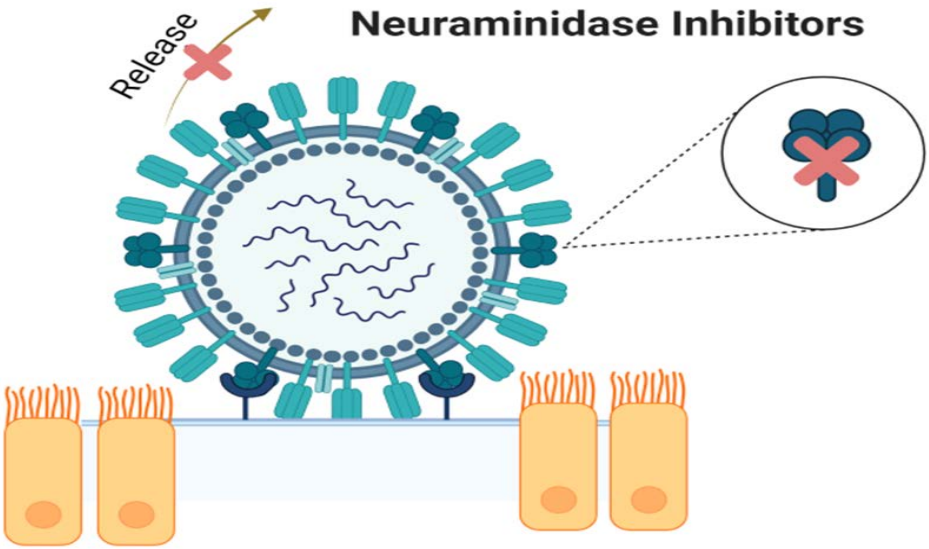

Figure 3. Neuraminidase Inhibitors

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 antivirals approved for the treatment of influenza in the United States, three, including 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 taken 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 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 taken intravenously [41]. Peramivir displays a low binding affinity to human plasma (<30%) [41]. However, 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 NA inhibitors are genuinely 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, although not in all patients [43–49]. While using NA inhibitors for prophylaxis is 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 [43]. Zanamivir produces fewer 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, possibly due to its intravenous route of administration [43]. The balance between their potential adverse effects and their potential benefits should be carefully weighed before drug administration.

Resistance to NA inhibitors is drug-specific; however, given the similar structure shared by the drugs in this class, resistance to one can affect the activity of others. Amino acid substitutions in either the NA catalytic site or the HA receptor binding site of influenza viruses can cause resistance to NA inhibitors to arise [50]. The H275Y amino acid substitution of the neuraminidase gene found in various influenza A viruses provides resistance towards oseltamivir and peramivir. Similarly, E119E/V (found in influenza A(H3N2) and A(H7N9)) causes resistance to oseltamivir and R292K causes 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 required [50].

4. M2 INHIBITORS (ADAMANTANES)

Adamantanes are a class of anti-influenza antivirals used specifically for treating type A influenza infections, although mass viral resistance has limited their recent use. There are only two members of this class, namely 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 on their mechanism of action [58]. M2 ion-channel inhibitors target the stage of viral uncoating. 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].

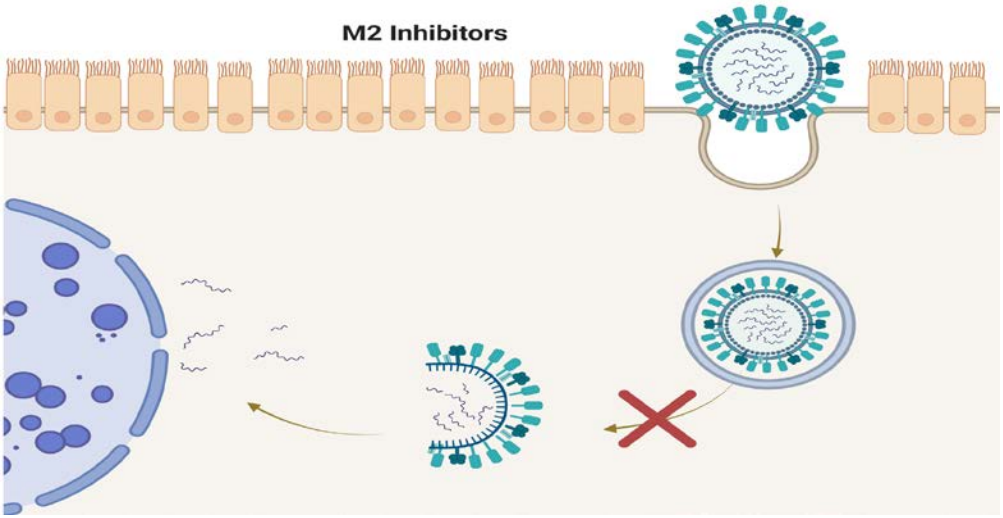

Figure 4. M2 Ion-Channel Inhibitors

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 dosages administered orally, that is, 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 within a large number of drug classes, including antihistamines and anticholinergic drugs, which further limits their usage [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 concentration in respiratory secretion, 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 resistance 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 noted to emerge as early as 2–4 days after the start

of the therapy in up to 30% of the patients infected with strains that showed susceptibility to either drug [60]. Many studies have demonstrated influenza resistance to this drug class [67–75]. Due to 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].

5. CAP-DEPENDENT ENDONUCLEASE INHIBITORS

The cap-dependent endonuclease, found within the RNA polymerase subunit of influenza viruses, plays a crucial role in facilitating the cap-snatching process during the creation of viral mRNA. This process is essential for the replication of the virus [76]. Baloxavir marboxil (xofluza), or baloxavir, was approved for the treatment of uncomplicated influenza first in Japan and then in the United States in 2018, followed shortly thereafter by several 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 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 the inhibition of cap-dependent endonuclease, baloxavir can inhibit viral replication for both influenza A and B viruses [5, 79] (Figure 5).

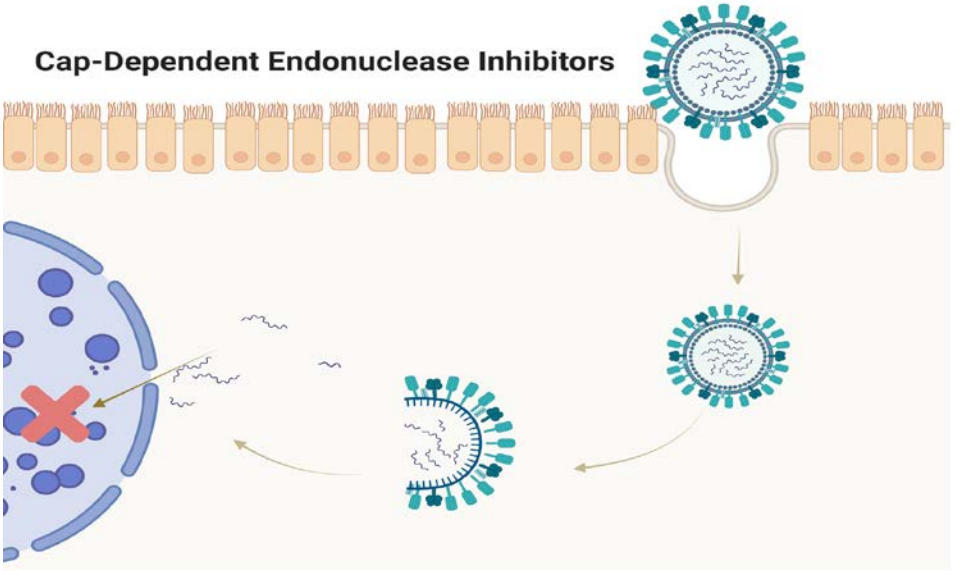

Figure 5. Cap-Dependent Endonuclease Inhibitors

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 co-administered CYP3A and UGT inhibitors, such as probenecid [5, 82]. Co-administration 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. Moreover, in patients with renal and hepatic impairments, baloxavir showed no altered pharmacokinetic properties [5, 82]. Baloxavir is suggested for use in patients 12 years of age and older who have been symptomatic for a maximum of 48 hours and only for acute uncomplicated influenza [5, 82]. In this regard, it is an inferior alternative to other antivirals that are also generally suggested for prophylaxis as well as influenza treatment. Baloxavir, however, is the preferred choice in patients where the use of NA inhibitors is contraindicated. Since it has a half-life of about 79 hours, baloxavir is given in a single-dose regimen [5, 82]. In this regard, it 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 substituted at I38 in the polymerase acidic protein, which resulted in reduced susceptibility to baloxavir [83]. Indeed, one clinical study that used this drug to treat influenza A(H3N2) reported that even after a single dose, a small subset of influenza patients developed resistance to it, with an overall rate of 19.5% resistance [77], while another clinical study showed resistance appearing between 8%-10% [84]. Interestingly, previous results reported a resistance rate of only 2.2%, however, the patients treated previously had contracted the 2009 A(H1N1) variant, the strain responsible for the 2009 pandemic [77].

In recent years, baloxavir resistance was only observed at the rates of 0.5% and 0.1% during the 2018-2019 and 2019-2020 flu seasons, respectively [85]. These results imply that baloxavir resistance varies across influenza strains and the drug remains a valid choice for treatment [86]. Additionally, when co-administered with oseltamivir, synergistic properties were shown between the two drugs. Moreover, resistance and drug-drug interactions were avoided [87–89]. Additionally, a recent study showed a lack of drug-drug interactions between baloxavir and NA inhibitors, though it failed to report improved clinical outcomes when compared to treatment plans consisting of a single antiviral [90]. These results suggest that if widespread viral resistance to baloxavir, NA inhibitors, or both occur in the future, co-administering baloxavir with an NA inhibitor may be the most effective treatment regimen to bypass resistance.

6. CONCLUSION

Antigenic drift in influenza strains allows these viruses to circumvent seasonal vaccines. Due to this fact, 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 the few antivirals currently available for the treatment of influenza do 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 antivirals has a unique mechanism of action, using a variety of anti-influenza antivirals may help to prevent resistance from cropping up quickly among influenza strains. In conclusion, the development of new antivirals, innovative combinations of existing treatments, and strategic co-administration with preventive measures, like vaccines, stand as our most effective strategies against the persistent threat of influenza.

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