Insights into the Development of Antiviral Therapies for Influenza and New Nanoparticle-based Approaches of Treatment

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Abstract

Influenza (the flu) is an annual respiratory illness that has resulted in a lot of societal burden. Presently, the first-line option to provide immunity against the virus is vaccination. However, due to the constantly mutating proteins on the virus, new vaccines have to be developed very frequently and this may not be a cost-effective option. Due to this, there has been an increasing interest in the usage of antiviral drugs for immediate treatment of influenza. One of the major disadvantages of using antivirals, however, is the emergence of strains that can become resistant to the available conventional drugs which is why extensive research is still being conducted in this area. This review provides an overview of the conventional drugs that have been in use, combination therapies, other advances in the creation of drugs that target various parts of the viral protein, and antibody-based therapies. Furthermore, the implications of a few nanoparticle-based approaches of treatment are also briefly discussed. To tackle the problem of drug resistance, combination therapies using the conventional drugs have been studied and have shown better results when compared to individual drug therapy. Antibody-based therapies with monoclonal antibodies that target the hemagglutinin protein of the virus have also shown encouraging results. With the advent of nanomedicine, new strategies for smooth drug delivery with Chitosan, Selenium and Silver nanoparticles are being investigated. Most of the results obtained from the studies discussed in the review show results of quick viral symptom resolution and indicate that the outlook in the field of upcoming antivirals is very promising. With additional studies, it can be determined the next steps for the development of influenza antivirals could be.

Introduction

Respiratory viruses such as influenza and the current SARS-CoV-2 infection have proven to be detrimental to the human population. Recent advances in vaccination and antiviral treatment strategies have helped to prevent the outbreak and harmful effects of these viruses. Although the influenza virus may have been around since ancient times, the Spanish flu pandemic that occurred in 1918-1920 was when this virus first proved to be a cause of global concern (Knobler et al, 2005). Approximately 500 million people were affected and around 50 million people died worldwide after being infected (CDC, 2019). Three major influenza pandemics followed the Spanish flu which included the Asian flu, the Hong Kong flu (Kilbourne, 2006) and most recently the swine flu in 2009. Currently, it is estimated that around 500 thousand people die every year due to this virus (Paget et al., 2019).

Influenza belongs to the class of *Orthomyxoviridae* and consists of four subtypes: influenza A, B, C and D. The recently discovered influenza D was found to affect only animals (Su et al, 2017). Influenza A is the most common form of the virus in humans and is primarily transmitted through inhaled droplets when people in close proximity cough or sneeze. Its common symptoms include high fever, cough, runny nose, fatigue and body aches (Taubenberger & Morens, 2008). Although it is presently a common annual and seasonal illness, it can lead to debilitating effects for a lot of people.

To curb the outbreak of influenza, standard vaccines such as inactivated virus and live attenuated virus vaccines were first developed to provide immunity (Ellebedy & Webby, 2009). Influenza viruses constantly evolve and undergo the process of antigenic drift and shift wherein mutations of the genes lead to different types of proteins being expressed on the virus. Vaccination strategies, therefore, have required intensive research and novel vaccines have to be developed very frequently due to this evolvement. Hence, there has been an emergence of antiviral drugs which work by targeting specific structures and phases in the replication cycle of the virus in order to overcome this issue. These drugs were developed for immediate treatment of people who were already infected with the virus and helped to combat the effects of the infection (Andrei, 2021). The main drugs that are currently prescribed and approved by the Food and Drug Administration (FDA) in the United States are neuraminidase inhibitors such as oseltamivir, zanamivir and peramivir and a polymerase complex targeting drug baloxavir marbaxil (FDA, 2020). The only two drugs approved by the European Centre for Disease Prevention and Control (ECDC) are oseltamivir and zanamivir (ECDC, 2022). In recent years, more drugs that target other parts of the virus (such as hemagglutinin) and host cells (such as sialic acid receptors) have also been explored (Yang et al, 2019). One of the biggest disadvantages of antiviral drugs, however, is that its prolonged use can lead to antiviral resistance. To combat this problem, therapies using a combination of antiviral drugs have been looked at.

Studies regarding drug delivery with nanoparticles have been an up-and-coming field and some research studies with nanomedicine for antiviral drugs for influenza have also been carried out. A nanoparticle-based approach of drug delivery ensures that the drug administered reaches the precise target in the body (Patra et al, 2018). Studies with antiviral drug-loaded selenium and metal nanoparticles show strong positive effects on the reduction of viral symptoms. Ongoing research currently being carried out in this area indicate that these nanoparticle-based approaches for tackling influenza are promising.

This review will focus on the various antiviral drugs that have been developed over the years in detail, their issues, combination therapies to counter these limitations and an interesting new nanoparticle-based approach of drug delivery and treatment. The future implications of this novel nanoparticle-based approach of antiviral drug delivery will also be discussed.

Influenza

Structure of the influenza virus

Influenza A viruses (IAVs) are the most common form of viruses that circulate among humans. IAVs can be spherical or tubular in shape and contain eight viral ribonucleoproteins (vRNPs). Each vRNP is made up of a negatively charged single stranded RNA bound with nucleoprotein (NP) and a heterotrimeric RNA polymerase complex consisting of PB1, PB2 and PA subunits. This RNA polymerase is responsible for the primary transcription and replication processes of the viral RNA. The vRNPs are enclosed and surrounded by a viral lipoprotein envelope which is derived from the host body itself. The M1 matrix protein is present on the inside of this viral envelope (Jung & Lee, 2020).

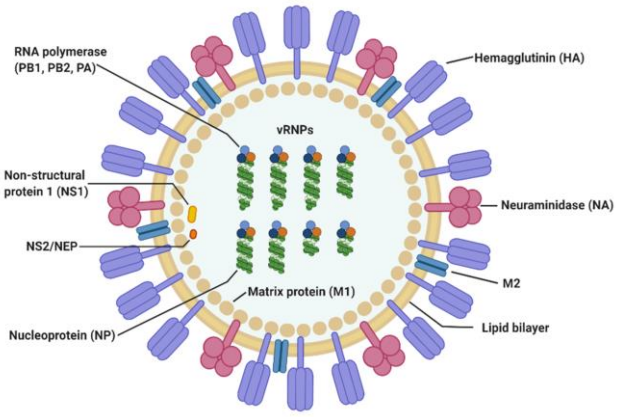

Figure 1: *Structure of an influenza A virus (IAV)*: 8 viral ribonucleoproteins (vRNPs) bound with nucleoprotein (NP) and RNA polymerase (PB1, PB2, PA). M1 matrix protein surrounds the vRNPS. Viral lipoprotein envelope (lipid bilayer) is embedded with neuraminidase and hemagglutinin spike glycoproteins and M2 ion channels. (Jung & Lee, 2020)

The influenza A virus expresses two spike proteins on its surface called hemagglutinin (HA) and neuraminidase (NA) (Figure 1). There are 18 types of HAs and 11 types of NAs that are currently known for IAVs (CDC, 2021). Depending on the number of different types of hemagglutinin and neuraminidase present, these IAVs are classified into different types such as the H1N1 virus which was the main cause of the Spanish flu and the swine flu pandemics. Some other commonly known IAVs include H5N1, H3N2, etc.

HAs and NAs are the most abundant spike glycoproteins and HAs aid in recognition of viral receptors on the host cells while NAs aid in releasing the virion from the host cell to infect adjacent cells (Kosik & Yewdell, 2019). Hemagglutinin consists of two sub-units: (i) HA-1 which forms the main globular head of the protein and contains the receptor-binding sites and (ii) HA-2 which forms the main part of the stalk and contains a fusion peptide. The viral protein envelope is also embedded with the less abundant M2 protein. The M2 protein functions as an ion channel and facilitates the entry of protons (Jung & Lee, 2020).

Entry of virus into host cell and replication cycle

The virus primarily enters the body through inhaled respiratory droplets and tries to gain entry into the host respiratory epithelial cells (Dou et al, 2018). The HA spikes on the viral envelope which are responsible for receptor recognition, initiate the process of virus attachment with sialic acid receptors present on the host cell membrane (Figure 2). After binding to these sialic acid receptors, the virus gains entry into the cell through endocytosis and this resulting virus is enclosed in an endosome.

Inside the cell, changes in the structure of the HA take place due to a low environmental pH (5.0) which leads to the exposure of the fusion peptide. This fusion peptide is crucial for allowing the viral membrane to fuse with the endosomal membrane. The low pH in the cell also helps to activate the M2 ion channels. When these channels are open, H+ protons rapidly enter, and form an acidic environment. This in turn

leads to the vRNPs being released from the endosome and entering the cytoplasm of the host cell (Samji, 2009).

From the cytoplasm, the vRNPs enter the nucleus with the help of importins, and the nucleus is where the process of viral RNA replication and transcription takes place with the help of the RNA polymerase heterotrimer complex (PB1, PB2 and PA). In the nucleus, multiple vRNA copies are made by replication and transported to the cytoplasm after which vRNP formation takes place. The transcription of viral mRNAs in the nucleus starts with a process known as cap-snatching in which nucleotides from a host RNA are 'snatched' to create a primer for the transcription process. This happens when the PB2 subunit binds to the 'capped' end of the host RNA after which the PA subunit - which has an endonuclease activity - cleaves 10-15 nucleotides from this RNA (Mifsud et al, 2019). With the help of these primers, viral mRNAs are transcribed. The mRNAs are exported from the nucleus back into the cytoplasm and complex translation steps take place here after which the IAV viral membrane proteins are formed and assembled (Dou et al, 2018).

The assembled proteins then bud out of the host cell and form new viral particles by using the host cell membrane as the viral envelope. The neuraminidase plays a crucial role in releasing the viral particles from the cell by breaking down the links between the virus and the cell receptors (Dou et al, 2018). The virus can then go on to infect other cells and spread to other parts of the body.

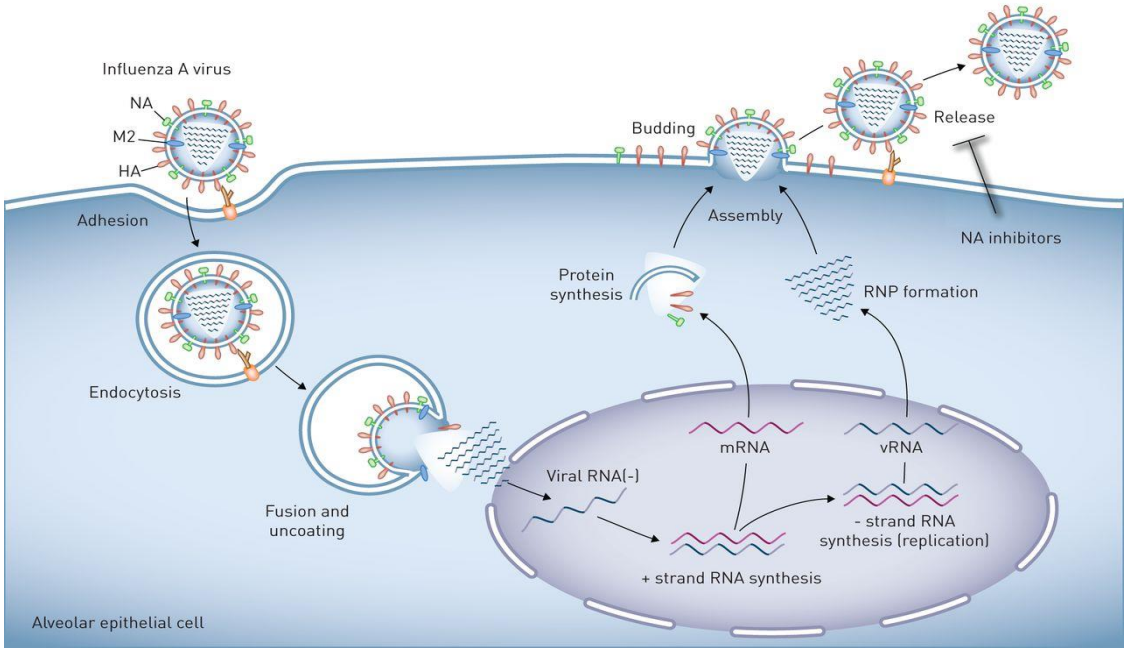

Figure 2: *Simplified overview of the influenza replication cycle*: Hemagglutinin (HA) allows the virus to adhere to the sialic acid receptors. Receptor-mediated endocytosis takes place, and the virus is enclosed in an endosome. Viral and endosomal membranes fuse after which M2 channels are activated and facilitate entry of protons acidifying the environment. Viral ribonucleoproteins (vRNPs) are released from the endosome into the host cell cytoplasm. vRNPS enter the nucleus and viral mRNA transcription and vRNA replication takes place after which they are exported back into the cytoplasm. Protein synthesis and RNP formation take place, and the virus particle is assembled. Virus buds out of the host cell and NA cleaves the link between receptor and virus for release. (Herold et al, 2015)

Antiviral therapies

1. Drugs that were/are in use for clinical treatment

The following sections discuss the drugs that are currently in use or were previously in use to treat the illness symptoms. The first antiviral drugs that were approved to treat influenza were M2 channel inhibitors and neuraminidase inhibitors. Further studies regarding the polymerase complex targeting drugs were also carried out which led to the development of newer and more effective forms of antivirals.

1.1. M2 inhibitors

Adamantane drugs such as amantadine and rimantadine function as M2 channel inhibitors. As previously mentioned, when the M2 channels are open, protons flow into the endosome and the acidified environment allows the vRNPs to be released. Thus, these drugs primarily function by blocking this proton transport. They can do this by interacting with the side chains of particular amino acid residues on the channels (Intharathep et al., 2008) which leads to the blocking of the channel pore. Amantadine and rimantadine were beneficial when they were first in use, but they are now not used as a first-line therapy for influenza due to the emergence of drug-resistant strains such as the 2009 H1N1 (CDC, 2021). Moreover, these drugs also resulted in toxic side effects such as nausea, dizziness and headaches which was another reason why their use was discontinued (Jefferson et al, 2006).

1.2. Neuraminidase inhibitors (NAIs)

Oseltamivir, zanamivir and peramivir are currently the first-line antiviral drug options for influenza and they function by blocking neuraminidase activity and thus, prevent the release of the virus particle to infect other host cells in the body. Oseltamivir is orally administered, zanamivir is inhaled as a dry powder and peramivir is intravenously injected as its availability is low and cannot be afforded for oral administration (Bassetti et al, 2019). Oseltamivir (OS) can be broadly distributed to the majority of the infection sites in the body such as the lungs and mucosa, and it is the most frequently used drug for influenza. In an early randomised controlled trial conducted by Treanor JJ et al, it was seen that the OS-treated group showed a 40% reduction in the extremity of the symptoms when compared to the placebo control group (Treanor et al., 2000). In another trial, it was found that giving OS to non-immunized people for 6 weeks had a 74% efficacy overall (Hayden et al., 1999) demonstrating that OS is indeed a beneficial antiviral drug. In 2007-2008, a large percentage of the circulating H1N1 strains which had a H275Y mutation in the NA protein, were resistant to OS (Garten et al, 2009). However, OS was found to be effective against the 2009 swine flu H1N1 strains which is why they have been in use even to this day. Studies have however shown that OS can also give rise to drug-resistant strains on treatment and prolonged use especially with people at higher risk of developing severe symptoms. It has been observed that in some of these immunosuppressed people, there was an emergence of OS-resistant H1N1 strains within 48 hours of starting treatment with OS (Inoue et al, 2010).

1.3. Polymerase complex targeting drugs

Drugs that target the polymerase complex of the virus were studied in more detail after M2 inhibitors were not found to be very effective. The only drug that is currently approved in Japan and the USA which targets the RNA polymerase is baloxavir. This drug functions by targeting the endonuclease activity of the PA subunit in the polymerase and prevents the cap-snatching process. Favipiravir and pimodivir were also studied as drugs that target the PB1 and PB2 subunit respectively, but they have not yet been completely approved for clinical treatment in many countries. The former is available in only some places in Japan since 2014 and is not widely prescribed due to its risk concerns with pregnancy (Mifsud et al, 2019).

1.4. Hemagglutinin inhibitors

Umifenovir (also commonly known as Arbidol) is an antiviral that has been used in Russia and China for many years, but the effects of this drug have not been extensively studied in other countries. It acts by targeting the HA and helps to stabilise it in the low pH environment and thereby, prevents the fusion process of the endosomal and viral membrane. In a study conducted in a hospital setting, it was found that in 0.3% of the umifenovir treated patients, pneumonia was found to be a complication due to the IAV. This percentage was low when compared to 23.7% of the patients who did not receive any therapy and had pneumonia. In OS-treated patients, however, none of the patients had this complication (Leneva et al., 2016) indicating that OS could have stronger effects. Currently, it is not clearly known whether umifenovir also gives rise to drug-resistant strains, but it is also deemed to be a potentially interesting area for research.

2. Combination therapies

Using combination therapies of the currently approved drugs may be an alternative in which the antiviral drug administration can be more effective especially for immunocompromised people who are at a higher risk of getting more severe symptoms (Bassetti et al., 2019). Moreover, using combination therapies can also help to overcome the problem of the emergence of drug-resistant strains to the conventional drugs.

2.1. Oseltamivir and baloxavir

In a study conducted by Fukao et al, three groups of mice that were inoculated with an influenza A strain, were treated with oseltamivir, baloxavir and a combination of OS and baloxavir respectively. It was found that the combination therapy of OS and baloxavir showed more reduced virus titres, a lower mortality rate and decreased pro-inflammatory cytokine production when compared to individual administration of either drug (Fukao et al., 2019). It is not possible to conclude if these studies can be translated to humans as detailed research has still not been carried out in this area but from these *in vivo* studies, it could be seen that the combination of OS and baloxavir may prove to be more effective than individual therapy with NAIs or polymerase complex-targeting drugs.

2.2. Oseltamivir and favipiravir

A few studies that have been carried out in mice indicate that the combination therapy of OS and favipiravir also showed a significant decline in mortality and cytokine production when compared to individual treatment with OS (Baz et al., 2018). One such study demonstrated that the mice had developed resistance to OS within 48 hours of treatment even though it was administered with favipiravir but stayed sensitive to the latter thus indicating that this combination should be considered for further research and clinical trials (Baz et al, 2018). In another study by Kiso et al, similar effects were observed and favipiravir was observed to be more beneficial as the mice remained sensitive to it throughout the course of the treatment but developed resistance to OS on prolonged treatment. Additionally, it was seen that stopping the treatment led to mice death. They concluded that the mice did show greater survival with the combination therapy for 28 days, but this strategy did not prevent the problem of OS-resistance (Kiso et al, 2018).

3. Other advances in antiviral therapies

Since the problem of drug-resistance with the currently approved drugs such as OS is a major concern, drugs that target other parts of the virus or host cell have been looked into in recent years. For example, there have been more studies done with drugs that target hemagglutinin, the M1 protein of the virus or the sialic acid receptors of the host cell.

3.1. Hemagglutinin-targeting drugs

Apart from umifenovir which is already in use in Russia and China, another potential HA-targeting drug that can be used to treat influenza is Nitazoxanide (NTZ). NTZ is used as a drug for a variety of other viruses but its potential role in influenza should be looked into in more detail to be used in clinical treatment. Unlike umifenovir, NTZ works by targeting the viral replication process. Its metabolite, tizoxanide, prevents the formation of HA protein after the translation process in the viral replication cycle and ultimately prevents the virus from completing the budding process and infecting other cells (Rossignol et al., 2009). A phase 2b trial carried out in the US with a placebo group and NTZ-treated group (two doses: 300 mg and 600 mg) indicated that the NTZ-treated group showed a reduction in symptoms by 36 hours when compared to the placebo group (Haffizulla et al., 2014). More phase 2/3 trials with NTZ are also currently being conducted to analyse its effectiveness (Koszalka et al., 2017).

3.2. Sialic acid-targeting drugs

The binding of the hemagglutinin to the sialic acid (SA) receptor of the host cell facilitates the entry of the virus to the cell and results in subsequent infection. Therefore, creating drugs that can inhibit this virus entry by blocking the SA receptors may be another promising strategy.

Sialic acid is a 9-carbon sugar that primarily consists of N-acetylneuraminic acid (Neu5Ac) which is linked to a galactose by an alpha-2,6 bond in humans. DAS-181 is currently the most promising sialic acid-targeting drug that is tested which functions to cleave the SA residues and thus inhibits the entry of the virus into the cell. A big advantage of using DAS-181 is that it can stop the virus from entering the airway epithelium itself and can thus have stronger effects on the reduction of virus symptoms (Zenilman et al, 2015). However, as discussed in a review by Heida et al, it is important to be aware of long-term effects of these sialic acid-targeting drugs as it is still not known whether removing these naturally occurring SA residues on the respiratory host cells can lead to other side effects since SAs also play roles in other biological functions of the body (Heida et al., 2021).

3.3. HA-targeting monoclonal antibodies

Antibody-based therapies have been in use for a long time to treat a wide range of infectious diseases whenever there are inadequate treatment opportunities (Beigel & Hayden, 2020). Thus, this may prove to be another way of tackling the problem of antiviral drug resistance.

As recently reviewed by Beigel and Hayden, although using polyclonal antibodies as a therapy option may be advantageous in a way due to its ability to target a wide diversity of antigen epitopes, they are not very antigen specific. Therefore, by developing monoclonal antibodies (mAbs) that target known epitopes may be more beneficial especially in the treatment of IAV as influenza epitopes have already been widely studied. Moreover, it may be easier to create mAbs in a large amount and they are also more cost-effective. Many mAbs that target HA have been tested in clinical trials against influenza, out of which some mAbs that are currently in their phase 2 trials will be discussed. They work in a similar way to umifenovir i.e, they inhibit the membrane fusion process by preventing structural changes in the HA fusion peptide from taking place (Tharakaraman et al, 2015).

An mAb that was developed is MHAA4549A and this targets a highly conserved epitope on the HA stalk. It can mainly help to neutralise H1, H2, H3, H5 and H7 strains (Nakamura et al., 2013, Beigel & Hayden, 2020). In a phase 2 study conducted in 100 participants with three different doses of MHAA4549A (400 mg, 1200 mg and 3600 mg), one standard dose of OS and placebo-treated groups, it was observed that the highest dose (3600 mg) of MHAA4549A was associated with a 97.5% decrease in the virus titre when compared to the placebo-treated group. This percentage was even higher than the 87% reduction observed in the OS-treated group. However, there were no significant differences between the placebo group and the MHAA4549A-treated groups who were administered with lower doses (McBride et al., 2017).

VIS410 is another mAb that targets the HA stalk that has also been used in phase 2 clinical trials and can neutralise H1, H5, H3 and H7 strains. In a phase 2 study by Hershberger et al in 2019, 150 patients randomly received one of two doses of VIS410 (2000 mg, 4000 mg) and their effects were compared to a placebo-treated group. The results declared that despite mild adverse effects such as gastrointestinal problems, VIS410 was deemed to be safe for use in patients and the lower dose of 2000 mg was more

successful in symptom resolution. To answer questions about whether these doses of mAbs are indeed safe and effective, further phase 2 and 3 trials need to be carried out (Hershberger et al, 2019).

Nanoparticle-based approach of antiviral drug delivery and treatment

Nanoparticles (NPs) are particles ranging from the size of 1 to 100 nm and they have been used in recent years in many fields of science including biomedicine. Due to their extremely small size, they are very versatile and can move freely in the body. One of the most common uses of nanoparticles in biomedicine is their ability to act as drug carriers or delivery agents as they can attach to the drug and target the precise site in the body (Patra et al, 2018). This is why the use of nanoparticle drug delivery to treat IAV has been tested in recent years. Apart from using nanoparticles for drug delivery, the bare NPs themselves can also be developed into drugs to treat an infection. The following sections will thus discuss the results of some studies that have been done with NPs for antiviral drug delivery and some bare NPs that have been evaluated to treat the virus.

Selenium nanoparticles

Selenium nanoparticles (Se NPs) are commonly known to show strong antibacterial and anticancer properties (Vahdati et al, 2020). To assess the antiviral efficacy of Se NPs, a study was performed by Li et al where H1N1 infected Madin-Darby Canine Kidney (MDCK) cells were used to assess the effects of Se NPs, oseltamivir and a combination of Se NPs and OS. It was observed that the combination of Se NPs and OS successfully prevented the infection and also resulted in lower toxicity when compared to individual treatment with Se NPs and OS (Li et al, 2017). In other studies also conducted by Li et al and Lin et al, Se NPs were used as drug carriers for other drugs like amantadine and umifenovir and all the results showed positive effects indicating that Se NPs may be a promising candidate for efficient drug delivery (Lin et al, 2017).

Silver nanoparticles

The first *in vitro* study conducted with silver nanoparticles (Ag NPs) as an antiviral therapy for influenza was conducted in 2009 by Mehrbod et al. Ag NPs have since then been studied as antiviral agents for a wide variety of viruses including the H3N2 influenza virus and the results have shown that they could prevent viral replication in many of the cases. In another recent study by Lin et al, zanamivir (ZNV) was loaded with Ag NPs to test their effects on the H1N1 infection specifically. As previously mentioned, zanamivir is a neuraminidase inhibitor that is currently still approved by the FDA for treating the influenza virus. Similar to the Se NPs experiment, H1N1 infected MDCK cells were used to assess the *in vitro* antiviral activities of individual treatments with Ag NPs and ZNV, and activity with the combined Ag@ZNV. From a neuraminidase inhibition experiment, it was noted that when the virus was treated with Ag@ZNV, lower neuraminidase activity was recorded when compared to treatment with Ag NPs and ZNV alone. Moreover, it was observed that with the treatment of Ag@ZNV, there was reduced apoptosis of the infected MDCK

cells. This was concluded to have happened due to the induction of p38 and p53 signalling pathways which down-regulated the ROS-mediated apoptosis of the cells after infection (Lin et al, 2017).

The combination therapy of Ag NPs and other antiviral compounds have demonstrated promising results. However, the exact mechanism of how Ag NPs act is still not known and therefore, a lot more research is needed in this area. Moreover, Ag NPs display high levels of cytotoxicity which when used in high concentrations, could lead to some issues.

Chitosan nanoparticles for siRNA-based therapy

Chitosan is a biopolymer derived from chitin and has many uses due to its non-toxic antiviral properties (Boroumand et al, 2021). Chitosan has been used as nanoparticles for delivery of a variety of drugs including novel siRNA-based drugs for influenza. These siRNA-based drugs work with the process of RNA interference (RNAi) which is an interesting strategy of preventing viral replication. RNAi works by silencing specific genes that are responsible for the infection in the post-transcription process. The process of RNAi is mediated by double-stranded RNA which are the small interfering RNA (siRNA) (Jamali et al, 2017). siRNAs have many advantages as they can be designed to target very specific conserved sequences of genes. Moreover, they are easier to design in a short amount of time and are cost-effective. A challenge that siRNA assessments have is the difficult of its delivery into the body which is why delivery of siRNAs using nanoparticles have been evaluated. Using chitosan NPs for siRNA delivery is useful as the siRNA can be administered nasally due to the mucoadhesive properties of chitosan (Barik, 2010).

The efficacy of siRNAs against influenza was studied both *in vitr*o and *in vivo* by Jamali et al, with siRNA loaded chitosan nanoparticles. Vero cell lines were used for the *in vitro* study while the chitosan/siRNA nanoparticles were administered nasally in mice, to ensure precise delivery into the lungs. It was reported that these NPs could prevent the growth of the virus in both of the cases (Jamali et al, 2017). siRNA-based drugs are currently in their phase 2 trials as they have shown statistically significant results in the reduction of virus symptoms and the use of the chitosan NPs for the delivery has also shown to be useful.

Discussion

The outbreak of influenza has caused widespread problems which include physical burden of the patients and economic burden to the healthcare system. This review provided an overview of the antiviral therapies that are being used to tackle influenza and the several developments that have been made in recent years to test a wide range of drugs which target different structures of the virus and the host cell. From M2 and NA inhibitors which have been tested in clinical trials, to the development of HA-targeting and sialic acid-targeting drugs, antiviral medication is an ongoing and promising area of research. To overcome the limitations of drug resistance and side effects on using these medications, combination therapies, antibody-based therapies and the use of nanoparticle-based approaches of treatment have been discussed briefly.

One of the primary problems of using antiviral drugs for the treatment of influenza is the rise of drug resistance. M2 inhibitors and NA inhibitors were the first class of antiviral drugs that were prescribed to patients but due to the emergence of drug-resistant strains and toxic side effects, the use of M2 inhibitors was discontinued. NA inhibitors (specifically OS) are the most commonly prescribed drugs these days for influenza and have shown positive effects such as a quicker reduction of virus titers and symptoms when compared to untreated groups. Studies have however documented that OS could ultimately also give rise to antiviral resistance and therefore, studies of combination therapies with polymerase complex targeting drugs have been done and are predicted to slightly overcome this problem. Other than the combination therapy of OS and baloxavir or OS and favipiravir, studies done with other combinations of drugs have also shown that using the combination strategy could indeed help to tackle the problem of resistance. It is also important to focus on targeting the parts of the viral protein that are conserved to combat the issue of resistance. One potential solution for this could be the previously discussed siRNA-based drugs that target and silence specific genes required for the expression of the viral protein which can further be studied and used as an alternative antiviral therapy.

Antiviral drugs must be taken within 48 hours of becoming sick with the illness for them to be effective. Thus, if they are taken after this time period, their effects may not be as strong which is yet another limitation of using antivirals. Using antivirals also has common toxic side effects which include gastrointestinal symptoms like diarrhea, vomiting and headache. In a lot of the cases, antiviral drugs have shown toxicity and therefore their long-term effects are still not clearly known. From the studies discussed in this review, it was seen that using Se NPs was a very promising strategy to prevent this problem as they helped in smooth delivery of the drugs OS, zanamivir and umifenovir but at the same time, did not result in toxicity. Therefore, finding more solutions to develop more drugs that can prevent toxic accumulation in the body is an important aspect to be further investigated.

Drugs such as nitazoxanide and DAS-181 are currently in the late phase of clinical trials as they have shown very encouraging results and mAbs such as MHAA4549A and VIS410 are currently in their phase 2 clinical trials. However, most of the clinical trials that have been conducted with mAbs and other antivirals till now have been on patients with acute and uncomplicated influenza. Therefore, the knowledge about the effects of these mAbs on more severe complications of influenza are still relatively unknown.

The use of NPs to deliver antiviral drugs has shown to tackle some issues of toxicity and NPs have the advantages of being very specific and moving freely through the body due to their small size. The few studies discussed in this review have tested the effects of combinations of NPs and traditional antiviral drugs. Further studies with different combinations of NPs and drugs can be done to determine whether using these NPs could also help to tackle the issue of resistance. Since nanomedicine with antivirals is a relatively new field, further *in vivo* research with NPs still needs to be done to confirm their effects in clinical trials. NPs may have a broader level of application for drug delivery due to their small size and high specificity which can be explored with more *in vivo* research.

To conclude, there are still a lot of studies needed to be done to confirm the long-term efficacy of antivirals and to overcome antiviral resistance. In order to do this, drugs can be created to target more conserved

parts of the virus, and the effects of various combinations of drugs can be analyzed. With the advancement in the new field of nanomedicine, antiviral therapies may prove to be very beneficial and could potentially also be used as a highly recommended and first-line option to fight viruses along with vaccination.

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