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Management of Influenza Virus Infections with Neuraminidase Inhibitors

Detection, Incidence, and Implications of Drug Resistance

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Contents

Abstract

Although influenza vaccination remains the primary method for the prevention of influenza, efficacy may be limited by a poor match between the vaccine and circulating strains and the poor response of elderly patients. Hence, there is an important role for antiviral therapy in the management of influenza. While amantadine and rimantadine have been available for the treatment of influenza in some countries for several years, they are only effective against influenza A viruses, they can have neurological and gastrointestinal adverse effects, and resistant virus is rapidly generated. Neuraminidase inhibitors, a new class of drug, are potent and specific inhibitors of all strains of influenza virus, and they have minimal adverse effects. The greatest benefit is seen in those patients presenting <30 hours after development of influenza symptoms, those with severe symptoms or those in high-risk groups. In addition to treatment of the infection, both drugs are effective prophylactically and have been shown to limit spread of infection in close communities, such as families and in nursing homes. No resistant virus strains have been isolated from normal individuals treated with zanamivir. Resistant virus can be isolated from approximately 1% of adults and 5% of paediatric patients with influenza treated with oseltamivir. However, infectivity of mutant viruses is generally compromised. Governments spend millions of dollars on influenza vaccination campaigns; however, once influenza virus is circulating in the community, vaccination cannot limit the spread of disease. A greater promotion of the use of neuraminidase inhibitors for the treatment and prevention of influenza could have a significant impact on limiting its spread. This could result in saving

millions of dollars, not only in direct costs associated with medical and hospital care, but also significant savings in indirect costs associated with the loss of productivity at work, school and home environments. For the benefit of all communities, there needs to be a greater awareness of the symptoms of influenza and the efficacy of neuraminidase inhibitors in disease treatment.

1. Influenza and Vaccination

Influenza is a severe infection of the upper respiratory tract responsible for significant morbidity and mortality throughout the world. It is estimated that in the US alone approximately 300 000 hospitalisations per year are attributed to influenza with up to 35 000 deaths.[1] Other viruses including parainfluenza virus, respiratory syncytial virus and Coxsackie viruses can all cause acute respiratory infections. Although there are several rapid diagnostic tests for influenza, most are complex and have low sensitivities (reviewed in Nicholson et al.[2]). However, onset of influenza illness is usually rapid, and when influenza virus is known to be circulating in the community, clinical diagnosis, based on fever plus two other symptoms including cough, sore throat, headache or myalgia, has been shown to be accurate in approximately 70% of cases. Diagnosis based on fever plus cough was shown to be accurate in up to 79% of patients in one study; however, a second recent study reported a predictive value of only 40–60% for these two symptoms.[3]

Although most symptoms of influenza are common to all ages, children tend to have higher fever than adults, as well as drowsiness and gastrointestinal symptoms, including abdominal pain, diarrhoea and vomiting. Elderly patients tend to have a higher incidence of lower respiratory tract symptoms, including sputum production, wheezing and chest pain.[4] Complications generally involve the respiratory tract, with acute bronchitis and pneumonia being the most frequent,[5] but young children and infants are at risk of acute otitis media, bronchiolitis and croup.[6] While the direct costs of primary medical care and hospitalisations run into billions worldwide, there is an even greater cost to the community with loss of productivity either in the workplace or home environments.

Vaccination remains the primary method for prevention of influenza and its potential complications. However, vaccination has its limitations. Influenza viruses undergo continual mutation, so that virus strains selected for the vaccine may be a poor match to the currently circulating strains; therefore, immunity may not be protective. In healthy individuals, the protective efficacy is estimated to vary between 70% and 90%,[7] whereas efficacy in the elderly or immunosuppressed can be as low as 40–60%.[8] In addition, when a severely ill patient presents in the clinician's office with suspected influenza or in the event of a pandemic, vaccination is not effective at the point of the outbreak, since it may take 2–4 weeks to achieve protective levels of immunity after vaccination. Hence, there is an important role for antiviral therapy in the management of influenza.

2. Anti-Influenza Therapy

There are two classes of drugs available to control the spread of influenza, each targeting different virus proteins. The first class, the adamantanes, blocks the ion-channel function of the M2 protein of the influenza virus. The second group, neuraminidase inhibitors, targets the neuraminidase of the virion.[9]

In order to understand how anti-influenza drugs work, it is necessary to understand how the influenza virus replicates. There are two types of influenza viruses that cause severe disease in humans, influenza A and B. Virions have two glycoprotein spikes on their surface, the hemagglutinin and the neuraminidase, which allow the virus to enter and leave cells. Hemagglutinin binds to cellular receptors that contain terminal sialic acids. Once bound to the receptor, the virus is endocytosed and transported into the cytoplasm. The virus M2 protein acts as an ion channel allowing the penetration of hydrogen ions from the endosome into the virus. The low pH leads to hemagglutinin unfolding and fusing with the endosomal membrane, releasing virus nucleic acid into the cell. Replication of the viral RNA and production of new virus proteins then occurs. Newly synthesized virions assemble and bud at the cell membrane. Since new virions all contain hemagglutinin, they will bind to the cellular receptors and to sugars on other virions as they bud from the cell. The second virus surface protein, neuraminidase, is an enzyme that removes sialic acids from the cellular receptors.[10] Since hemagglutinin can no longer bind to the cell, this allows release of progeny virions. In addition, removal of sialic acid from virus proteins prevents newly formed virions from binding to each other and aggregating. Neuraminidase may also play a role in facilitating movement of the virus through the mucus layer in the respiratory tract.[11]

2.1 Adamantanes

The adamantanes block the function of the M2 protein at low concentrations, and at very high concentrations act indirectly on hemagglutinin, preventing fusion of the virus and cell membranes.[12] Amantadine has been available in the US for prophylax-

is and treatment of influenza since 1976 and rimantadine has been available since 1993. However, these drugs have not been approved for use in all countries. Although these drugs may be the cheapest option for treating influenza, they also have a number of limitations in their use. They are only effective against influenza A viruses, since influenza B viruses do not have an M2 protein. In addition, 10–30% of patients suffer from intestinal and CNS adverse effects; this can exacerbate problems in the elderly, who may already be disoriented.[13] Furthermore, resistant virus is readily generated in up to 30% of patients.[14] Mutant viruses are still virulent and readily transmitted,[15,16] and can be isolated from patients in the community who have never been exposed to these inhibitors.[17,18]

2.2 Neuraminidase Inhibitors

Neuraminidase inhibitors are highly potent and specific inhibitors of both influenza A and B viruses. They were designed using a new approach to drug development based on understanding the structure of the target molecule and how it interacts with its substrate. The determination of the 3-dimensional structure of the influenza neuraminidase in complex with sialic acid led to the design of zanamivir.[9] Zanamivir interacts with a group of amino acids in the active site of neuraminidase, which are conserved in all influenza A and B strains. Zanamivir blocks the action of neuraminidase, which prevents the cleavage of sialic acid on the cell receptors, thus preventing release and spread of the newly formed virions. Since neuraminidase activity is also thought to facilitate the passage of virus through mucus, blocking neuraminidase activity may also prevent the virus from reaching respiratory cells. Zanamivir is administered by oral inhalation, achieving high concentrations of drug in the respiratory tract, the site of virus replication at a dosage of two inhalations of 5mg each (total dose 10mg) twice daily for 5 days. Based on the efficacy of zanamivir, a second inhibitor, oseltamivir, was subsequently developed.[19] Oseltamivir is taken orally as the ethyl ester prodrug, oseltamivir phosphate, which is converted by hepatic esterases into the active form, oseltamivir carboxylate. The drug is administered at a dosage of 75mg twice daily for 5 days.

Two other neuraminidase inhibitors have been developed; however, neither is being pursued. Peramvir (BCX 1812, RWJ 270201) originated by Biocryst Pharmaceuticals [20] was an effective inhibitor *in vitro*; however, significant improvements in patients' outcomes were not observed in clinical trials. Abbott was also developing a neuraminidase inhibitor (A 315675)[21] which displayed *in vitro* inhibitory activity.[22] However, this drug has not been taken into clinical trials.

There have been recent reports of enhanced potency of multivalent zanamivir by Sankyo in Japan[23,24] and Biota in Australia,[25] where a single dose of drug may be sufficient for protection.

3. Clinical Trials

3.1 Treatment

3.1.1 Zanamivir

Randomized, double-blind, placebo-controlled trials have been undertaken for both zanamivir and oseltamivir. A number of articles have reviewed and summarized data from the clinical trials involving both drugs.[26-30] The primary clinical endpoint was the length of time to alleviation of symptoms (fever, myalgia, headache, sore throat or cough) for at least 24 hours. Reduced virus shedding was also observed with both drugs. However, it has recently been reported that more than 10^3 infectious units/mL of influenza virus could still be isolated from some children treated with oseltamivir, even after 5 days of treatment.[31]

For zanamivir, a mean 1–1.5 days of earlier symptom relief was observed for all patients, including adults and paediatric patients compared with inhaled placebo[32]. Despite a concomitant reduction in the severity of symptoms, the perception among many clinicians and public is that 1-day of symptom improvement is not significant. However, when the data was analysed among different risk groups, more significant results were demonstrated. Those that derived the greatest benefits (2–3 days of symptom relief) were high-risk patients, those presenting with severe influenza-related symptoms or those who had symptoms for <30 hours. Older patients, aged >50 years, presenting with severe symptoms derived up to 7 days of benefit, as in this group, untreated patients experienced up to 11.5 days of symptoms.[32]

In addition to earlier symptom relief, patients returned to normal activities sooner, and there was a significant decrease in the use of symptom relief medication and antibacterials. Adverse effects were no different between the placebo- and zanamivir-treated groups. Since zanamivir has low systemic availability, no dosage adjustment is necessary for patients with impaired renal function. While there were reports of bronchospasm post-release, trials specifically targeting patients with mild-to-moderate asthma or COPD showed that zanamivir was an effective treatment and had a similar safety profile to the inhaled lactose placebo.[33] There were no adverse effect on pulmonary function; however, the pack insert recommends that patients should have a fast-acting bronchodilator available. Interestingly, there were also five cases of bronchospasm reported in the oseltamivir clinical trials:[34] one in the placebo group and four in the oseltamivir group, suggesting

that bronchospasm may be a symptom associated with influenza infection.

Post-marketing surveillance of more than 15 000 patients showed that symptoms improved within 2 days of initiation of treatment with zanamivir in >70% of patients and two-thirds of patients returned to normal activities within 3 days.[35] Despite the low incidence of zanamivir prescribing by clinicians, due to the perception that the inhaler is difficult to use, particularly by the elderly,[36] post-marketing surveillance showed that 89% of patients aged >65 years were comfortable with using the inhaler.[37,38]

3.1.2 Oseltamivir

For oseltamivir, a benefit of 1–1.5 days of earlier relief of symptoms was observed for all patients, and a greater benefit of 2 days was observed for those presenting at less than 24 hours after onset of symptoms.[26-28,30] Patients in the oseltamivir treatment group returned to normal activities sooner, and there was less use of antibacterials and relief medication compared with the placebo group. Likewise, children treated with oseltamivir also experienced a 1.5-day benefit of symptom relief, returned to normal activities sooner, had a reduction in prescription of antibacterials and fewer complications, including otitis media,[39] than the placebo control group. Separate data on the efficacy of oseltamivir treatment of high-risk patients have not yet been published. Adverse effects included nausea and vomiting, with 17% experiencing transient nausea in the oseltamivir group compared with 7% in the placebo group.[40] Effects were primarily in the first 48 hours of treatment and could be partially reduced by taking the drug with food. A study in experimental influenza found that administration of oseltamivir with food reduced the incidence of gastrointestinal disturbances from 31% to 7%.[40] Dosage adjustment to 75 mg/day is needed for patients with creatinine clearance <30 mL/min. For patients with clearance rates <10 mL/min, caution is advised even with the lower dose of oseltamivir (package insert). There has also been a recent report of death in juvenile rats treated with high doses of oseltamivir.[41] Brain levels of oseltamivir were 1500-fold higher than those of adult animals exposed to the same dose. It is hypothesized that immaturity of the blood-brain barrier caused the toxicity. The US FDA has therefore instructed Roche to include a warning in the package insert, indicating that oseltamivir should not be administered to children <1 year of age. Oseltamivir is administered as syrup to paediatric patients.

Co-infection with *Streptococcus pneumoniae* and influenza can have a synergistic effect on disease severity, leading to excess mortality.[42] In a mouse model, it was demonstrated that the stripping of sialic acid from the lung by influenza virus neuraminidase potentiated development of pneumonia by exposing receptors for pneumococcal adherence. Oseltamivir improved survival of mice, independent of viral replication and morbidity from influenza.[42] Thus, treatment of influenza with neuraminidase inhibitors may play an important role in preventing exacerbation of pneumonia.

3.1.3 Efficacy Against Different Isolates

Neuraminidase inhibitors are effective against all subtypes of neuraminidase, which is of critical importance in treating any potential pandemic strain of influenza virus. Testing of approximately 1000 human H1N1, H3N2, H1N2 and influenza B viruses, has demonstrated that zanamivir is slightly more effective against N1 and influenza B viruses, and oseltamivir is slightly more effective against N2 viruses.[43] In addition, neuraminidase inhibitors have been shown to be specifically effective against neuraminidase from the virus responsible for the 1918 pandemic,[44] as well as against the H5N1 and H9N2 avian virus strains responsible for the 1997–1999 outbreaks of avian influenza in Hong Kong.[45,46] The highly pathogenic avian H5N1 viruses, which were circulating in Asia during the period 2003–4 were also sensitive to neuraminidase inhibitors,[47] but interestingly were resistant to the adamantanes.[48] There is a perception that multiple organ failure observed in the few humans who have died as a result of an H5N1 infection, is due to systemic spread of virus and multiplication in the peripheral organs. Hence, only a drug that acts systemically would be effective. However, post-mortem examinations of humans infected with the chicken virus strains have failed to find virus anywhere other than in the respiratory tract and lungs.[49] Similarly, animal models of chimpanzees infected with the avian influenza viruses show that although the animals die of organ failure, virus is only detected in the respiratory tract and lungs.[50] Destruction of other organs appears to be due to high levels of cytokines released in response to influenza virus infection. Since the infection is still spread by the respiratory route, and limited to the respiratory tract, inhaled zanamivir should be just as effective as oral oseltamivir.

3.2 Prophylaxis

In a randomized double-blind, placebo-controlled trial, participants began 4 weeks of zanamivir at the start of an influenza A outbreak. Zanamivir was 84% effective in preventing laboratory confirmed illness with fever.[51] Interestingly, many patients were apparently infected asymptomatically since many developed an immune response without associated symptoms, thus providing protection from reinfection. In a study evaluating prevention of influenza in families, where both index cases and family contacts were treated, zanamivir was 79% effective in preventing influenza in contacts. Zanamivir was effective against both influenza A and

B, and the index cases had symptoms for 2.5 days less than placebo-treated controls.[52] In a second trial where the index case received relief medication only, zanamivir treatment of household contacts had an 81% protective efficacy against laboratory-confirmed influenza, and protective efficacy was similarly high against both influenza A and B.[53] Zanamivir was also effective in a nursing home outbreak, where despite a 90% vaccination rate there was an outbreak of A/Sydney/05/97 H3N2-like virus, a component of the vaccine. Amantadine therapy did not control the spread, and amantadine-resistant viruses were isolated. In the 2 weeks after zanamivir prophylaxis, no new cases of influenza were confirmed.[54]

Prophylaxis studies with oseltamivir in patients challenged with influenza A virus showed that oseltamivir was 61% effective in preventing influenza infection based on serology, and no patient shed virus.[40] Oseltamivir did not reduce infection rates in the influenza B virus challenge, although it did reduce the virus titre and duration of shedding.[55] A further trial where patients were treated for 6 weeks during a local influenza outbreak demonstrated a protective efficacy of 74%.[56] In a household contacts trial where the index case was untreated, the overall protective efficacy against clinical influenza was 89% for individuals and 84% for households.[57] In what was thought to be an influenza B virus outbreak in a nursing home, oseltamivir prophylaxis reduced the incidence of influenza infection by approximately 50%, compared with a home where no treatment was used.[58] In a frail elderly population, oseltamivir prophylaxis resulted in a 92% reduction in the incidence of laboratory-confirmed clinical influenza compared with placebo.[59] Consistent with the reduced efficacy of vaccination in elderly patients, 12 of 13 patients who became infected in the placebo group had been vaccinated.

4. Drug Resistance

Since neuraminidase inhibitors were designed at the molecular level to interact only with the active site of the virus neuraminidase, mutations altering the ability of the drug to bind are likely to have detrimental effects on the ability of the virus neuraminidase to function normally. Because of the differences in the chemical structures of zanamivir and oseltamivir, they bind slightly differently in the enzyme active site. This means that mutations may affect the binding of one, but not necessarily both drugs. Decreased sensitivity to the inhibitors can be generated in cell culture in the laboratory, but only after extensive exposure of viruses to the drugs.[60] Mutations *in vitro* are found not only in neuraminidase, as expected, but also in hemagglutinin.

4.1 Lack of Naturally Occurring Resistance

As discussed above, the influenza M2 inhibitors have a very poor resistance profile, rapidly generating resistant viruses, which are virulent and transmissible. Furthermore, resistant virus can be isolated in untreated patients in the community. Because neuraminidase inhibitors are a new class of drug, there are concerns about the potential for the emergence and spread of resistant variants. The regulatory authorities have requested surveillance be carried out to monitor both the potential drift in the baseline sensitivity of isolates and the emergence of resistance in individual isolates. The global neuraminidase Inhibitor Susceptibility Network (NISN)[61] was established to coordinate testing of clinical isolates to determine baseline sensitivities of isolates prior to introduction of the inhibitors into clinical practice, as well as post-release. Of approximately 1000 isolates tested, collected prior to drug release based on sensitivities of their neuraminidases, there was no evidence of naturally occurring resistance to either drug in any of the isolates.[43] It is proposed that post-release monitoring should be carried out for 5 years.

4.2 Resistance due to Hemagglutinin Mutations

Although hemagglutinin mutations are the predominant mutation in cell culture, their role in altered sensitivity of clinical isolates is not known. Mutations in hemagglutinin are located in and around the regions of the molecule involved in binding to the cellular receptor. Reduced affinity of hemagglutinin for the sialic acid means the newly formed virions do not bind as strongly to the cell receptors, and they can therefore still elute even with reduced neuraminidase activity. Thus, hemagglutinin mutations[60] result in decreased sensitivity to all neuraminidase inhibitors. Decreased sensitivity due to hemagglutinin mutations is demonstrated by the growth of virus in concentrations of either drug known to inhibit replication of wild type virus. However, the altered binding is specific to the type of cell and receptor. Hence, a human clinical isolate may not have altered sensitivity in the Madin Darby Canine Kidney (MDCK) cells most commonly used to grow influenza in the laboratory. One virus containing a hemagglutinin mutation at Arg198Thr (as well as an neuraminidase mutation; see section 4.3) was isolated from an immunocompromised child treated with zanamivir.[62] However, the virus was sensitive to zanamivir in the MDCK cells; hence, its role in *in vivo* resistance is not clear. An hemagglutinin mutation, Ser262Asn was detected in a child in a recent Japanese study,[31] in conjunction with a neuraminidase mutation (Arg292Lys); however, its role in resistance has not yet been confirmed.

Two H3N2 viruses with hemagglutinin mutations at Arg229 were isolated from untreated patients.[63] Mutations at this residue

in hemagglutinin conferred decreased sensitivity to both neuraminidase inhibitors in H1N9 viruses passaged *in vitro*.[64] Although both the H1N9 and H3N2 viruses had reduced sensitivity to neuraminidase inhibitors in MDCK cells, the H1N9 mutant viruses had reduced sensitivity in mice,[60] whereas the human H3N2 isolates were sensitive in ferrets, which are thought to have receptors more like those in humans. Hence, these mutations may have no effect on drug sensitivity in humans. Since the cells used in the laboratory have different types of receptors compared with human respiratory cells, there is currently no suitable cell for assessing the role of hemagglutinin in the development of resistance in human clinical isolates. One group is attempting to make an MDCK cell which has a higher density of human like sialic acids;[65] however, this has not yet been tested with a panel of known resistant hemagglutinin mutants.

4.3 Resistance due to Neuraminidase Mutations

Drug resistance due to mutations in neuraminidase are detected by screening viruses in an enzyme inhibition assay. To date, this is the only accepted method of determining drug resistance in clinical isolates. Neuraminidase mutations can render the enzyme from 10- to 10 000-fold less sensitive to the inhibitors. Mutations in neuraminidase can be both drug and virus neuraminidase subtype specific.[60] Both zanamivir and oseltamivir are based on the structure of a product of sialic acid generated during catalysis, 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (DANA) [figure 1]. However, zanamivir has only a single substitution of a guanidinium group at the 4′ position on the sugar ring. Oseltamivir has several differences, since it contains a cyclohexene ring instead of the sugar ring, and has two substitutions on this ring. It has a substitution of an amino group at the 4′ position and a large pentyl ether group replacing the glycerol side chain at the 6′ position. Binding of oseltamivir requires the movement of amino acids in the active site of neuraminidase to accommodate the larger side chain, whereas zanamivir does not. Because of the differences in their chemical structures, there are specific mutations that affect the binding to each of the inhibitors. For example, mutations preventing the movement of the amino acids in the active site confer a higher level of resistance to oseltamivir, whereas those that affect the interaction with the guanidinium group confer greater resistance to zanamivir. A virus with an N9 neuraminidase has been used as a model virus for resistance analysis *in vitro*, since the 3-dimensional structure of neuraminidase is known. Analysis of how single mutations affect drug binding at the structural level has provided valuable insights into the mechanisms of drug binding. It has also led to the hypothesis that a general strategy for drug design when the target has a high mutation frequency is to design the inhibitor to be as closely

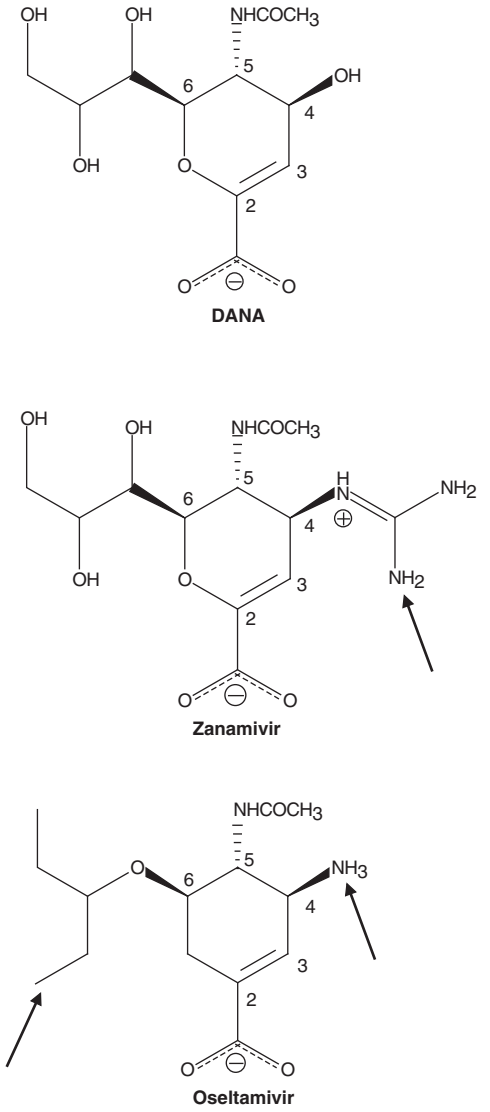

Fig. 1. Chemical structures of 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (DANA), zanamivir and oseltamivir. Arrows point to where the inhibitors are different from the natural intermediate, DANA.

related as possible to the natural ligands of the target. This means that the greater the difference between the inhibitor and the natural substrate, the greater the possibility that mutations can arise that affect binding of the inhibitor without affecting binding of the natural substrate.[66]

There has not yet been any documented case of clinical resistance in a previously healthy individual treated with zanamivir. The only resistance seen after zanamivir treatment has been in an immunocompromised child after a prolonged infection with influenza B and treatment with zanamivir for 15 days.[62] This virus had a mutation at Arg152Lys in neuraminidase, and Arg198Thr in hemagglutinin, neither of which have been seen *in vitro*. This virus

is less sensitive to both zanamivir and oseltamivir in enzyme inhibition assays.[43]

Mutations in neuraminidase causing resistance to oseltamivir have arisen both in challenge studies and in patients with naturally acquired infections. Rates of resistance are estimated to be around 1% in the adult population and 5% in pediatric patients.[39,67] There is, however, a recent report of resistant virus being isolated from 9 of 50 (18%) children treated with oseltamivir in a Japanese study.[31] In addition, wild-type virus was isolated from 24 patients despite several days of oseltamivir treatment.

Mutations seem to be specific to the subtype of influenza virus. The most common mutation in N2 viruses is Arg292Lys, which gives 1000- to 10 000-fold resistance to oseltamivir in an enzyme sensitivity assay, and 10- to 100-fold reduced sensitivity to zanamivir. Although this mutation has been generated after passage of the N9 and N2 viruses in oseltamivir or zanamivir in cell culture,[60] it has never been seen in clinical isolates after zanamivir treatment.

A second neuraminidase mutation Glu119Val, which gives 100- to 500-fold resistance,[31,68] has been selected after exposure to oseltamivir, *in vitro* in the N9 virus, and from clinical isolates in N2 viruses.[31,67,69] Mutant viruses are resistant only to oseltamivir. Although viruses carrying mutations at Glu119 to Gly, Ala or Asp have been generated after passage in zanamivir *in vitro*,[70] no viruses with mutations at Glu119 have been isolated in patients treated with zanamivir.

A His274Tyr mutation has been isolated only in N1 viruses. This mutation confers about 400- to 600-fold resistance,[68] and has been seen in both cell culture passaged virus and in clinical isolates after exposure to oseltamivir. Resistance is specific to oseltamivir. There is a report of an oseltamivir-resistant His274Tyr neuraminidase mutant virus being isolated from an immunocompromised patient treated with amantadine, oseltamivir, rimantadine and zanamivir.[71] The patient continued to shed the resistant virus for at least 12 months. This virus was still susceptible to zanamivir.

A recent report from a paediatric study in Japan also describes an Asn294Ser mutation in an H3N2 virus. This mutation has not previously been seen either in laboratory generated resistant viruses or in clinical isolates and conferred about a 300-fold decrease in sensitivity to oseltamivir.[31]

Resistance in influenza B viruses has not been seen in previously healthy individuals. However, a virus with a drug-resistant neuraminidase has been isolated from an oseltamivir-treated immunocompromised patient infected with influenza B virus. TheAsp198Asn mutant neuraminidase has reduced sensitivity only to oseltamivir, not zanamivir.[72] This mutation has not been seen *in vitro*.

Since all mutations so far are located in or around the active site of the enzyme, the mutant neuraminidases have demonstrated either reduced stability, reduced enzyme activity, or reduced infectivity *in vitro* or in animal models.[60,73,74]

Poor infectivity of mutant viruses has been shown to affect virus transmission in animal models.[73] Ferrets infected with an A/Sydney/5/97 (H3N2) clinical isolate carrying the Arg292Lys mutation did not transmit mutant virus to any contact animal. Unexpectedly, wild-type virus was isolated in contact animals; apparently the mutant virus underwent reversion in the original infected animal. Thus, it could be theoretically possible that oseltamivir treatment could generate a resistant mutant, which may result in continued spread of a wild type revertant virus after cessation of treatment.

However, despite the viruses being compromised in their growth *in vitro*, a more recent publication has demonstrated transmission of both the Glu119Val and His274Tyr mutant viruses in ferrets.[75] The Glu119Val virus grew to similar titres in both the donor and contact animals. Although 100-fold more virus was needed to infect the donor animals with the His274Tyr mutant virus, and virus replication was delayed by 1 day, virus grew to the same titres in both donor and contact animals. All mutant viruses retained their genotypes after transmission.

To date, there has been no documentation of the spread of a resistant virus to a contact in humans, in contrast to that observed with amantadine-resistant viruses during amantadine treatment. However, these recent results suggest that, despite the compromised nature of these viruses, there are concerns about their transmissibility in humans.

5. Post-Marketing Data

While there are many data published on the clinical trials with neuraminidase inhibitors, Vogel[76] recently published an evaluation of the use of neuraminidase inhibitors in the normal clinical setting. Fifty-five patients treated with zanamivir and one treated with oseltamivir observed a rapid drop in temperature of 1.7°C within 12 hours. A 96-year-old patient demonstrated an impressive improvement within 6 hours. The duration of illness was decreased by 45% and administration of antibacterials was decreased by 32% compared with patients presenting with influenza infection prior to the availability of neuraminidase inhibitors. Fibrinogen levels were also lower in patients treated with neuraminidase inhibitors than in patients without specific antiviral therapy. The author suggested that the prevention of high fibrinogen levels by early antiviral treatment may have an impact on the incidence of myocardial infarction in patients with established coronary atherosclerosis.

In another study, military conscripts treated with zanamivir for naturally acquired influenza virus infection had a rapid reduction in virus load, up to 2.5 days of reduction in median time to alleviation of symptoms, and 43% reduction in complications requiring antibacterials.[77] As well as being of benefit to the treated patient, reduced viral shedding could result in a lower rate of infection spread, which would be important in a closed community. The Diskhaler™ [1] was rated as easy or very easy to use by 99% of patients at the end of therapy.

Faced with the difficulty of knowing whether the patient has influenza and if antiviral therapy is appropriate, analysis by Smith and Roberts[78] found that antiviral treatment of influenza without rapid testing is economically reasonable in febrile patients with typical symptoms during the influenza season. The selection of antiviral therapy depended on the likelihood of influenza A virus infection, patient age and differences in medication cost. Zanamivir treatment was considered the most effective strategy, minimising both quality-adjusted days lost and illness days. In the UK, the National Institute for Clinical Excellence has recommended the use of neuraminidase inhibitors for elderly and at-risk patients who present with influenza-like illness within 36 hours of symptom onset. As there was a concern that the cost of neuraminidase inhibitors could have a significant impact on the national health budget, Da Silva et al.[79] sought to determine, during a confirmed influenza outbreak, the proportion of eligible patients who presented in time to benefit from treatment with a neuraminidase inhibitor. Only 20% of such patients, rising to 47% in out-of-hours centres, consulted in time to benefit from treatment. Since children play a critical role in the spread of influenza in the community and households, targeting children for treatment and prophylaxis with the inhibitors could play an important role in further limiting the spread of influenza in the community.

6. Effect on Immunity

Although vaccination is the primary method for prevention of influenza, once there is an outbreak in an unvaccinated community, vaccination cannot provide protection sufficiently rapidly. Neuraminidase inhibitors do not interfere with the development of an immune response to killed influenza virus vaccines;[80,81] therefore, they should have the potential to provide protection during the 2- to 4-week period before full immunity is induced. Since the drugs do not prevent the initial virus replication, but prevent subsequent virus release, when used therapeutically, an immune response develops in treated patients. Even in patients treated prophylactically, although asymptomatic, many develop an immune response, thus providing protection against reinfection.

7. Conclusions

Zanamivir and oseltamivir are two potent and specific inhibitors of all strains of influenza virus. Unfortunately, the focus on the number of days of symptom relief provided has detracted from the importance of their impact on the severity of the symptoms. The greatest benefit is derived if patients are treated as early as possible and for those presenting with severe symptoms or in high-risk groups. Both are effective therapeutically and prophylactically; approval for ages and use varies in different countries.

Zanamivir has some potential advantages over oseltamivir including acting rapidly, as it is delivered at the site of infection, having no adverse effects compared with placebo, and no documentation of resistance in previously healthy patients. In contrast, after oseltamivir administration there is a delay of 3–4 hours to reach therapeutic levels at the site of infection, some patients experience nausea and vomiting and there is a low level of resistance observed. However, psychologically, oseltamivir has the greatest advantage, being orally administered. Despite surveys showing satisfaction of patients using the Diskhaler™ device for zanamivir,[37,38] it is far easier to prescribe a tablet, and consequently oseltamivir has by far the greatest share of the market. For young children and for infirm or frail elderly, oseltamivir would be the drug of choice.

With the ever-present threat of a pandemic there is a critical role for the immediate availability of neuraminidase inhibitors to limit spread. However, unless there is wider acceptance in the community of a role for these drugs in the treatment and prevention of influenza, there will not be sufficient stocks available to have any impact. Governments are willing to spend hundreds of millions of dollars on vaccination and in advertising the vaccination campaigns, yet their reluctance to fund neuraminidase inhibitors means that they are not seriously addressing approaches to limiting spread of influenza once an outbreak has occurred. If governments were serious about trying to limit the impact of influenza in the community, they should promote greater public awareness of the symptoms of influenza and the availability of the drugs for both treatment and prevention.

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1 The use of trade names is for product identification purposes only and does not imply endorsement.

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