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Influenza Virus Resistance to Neuraminidase Inhibitors: Implications for Treatment

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Abstract Oseltamivir and Zanamivir are the two main Neuraminidase inhibitors used for the treatment of Influenza. Oseltamivir resistance has been identified in non-pandemic influenza viruses, as well as H1N1 pandemic Influenza A viruses. Resistance is associated with increased morbidity, and poorer outcomes in severely immunocompromised hosts. Newer neuraminidase inhibitors, increased vaccination and combination therapy may be alternatives for the treatment of Influenza in this setting.

Keywords Influenza · Neuraminidase inhibitor resistance · Oseltamivir · Zanamivir · Laninamivir · Peramivir · H274Y mutation · H1N1 pandemic influenza

Introduction

Influenza viruses belong to the family Orthomyxoviridae [1]. Influenza epidemics occur seasonally in winter in the United States (US). The Centers for Disease Control and prevention (CDC) estimates that between 1976 and 2007, the annual incidence of Influenza related deaths from respiratory and circulatory causes ranged from between 3,349 in 1986 to 1987 to as high as 48,614 in the 2003 to 2004 season [2]. Approximately 90% of these deaths were in people 65 and older [2]. Worldwide, the number of cases is estimated to be between 3 to 5 million yearly, with up to 250,000 to 500,000 deaths [3]. The Neuraminidase Inhibitors (NAI) and Adamantanes form the basis of treatment and prevention of Influenza.

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Neuraminidase Inhibitors

Hemagglutanin and Neuraminidase are the two main glycoproteins on the surface of Influenza A and B viruses. Sialic acid containing glycoproteins serve as host cell receptors for Influenza viruses; Neuraminidase cleaves the terminal sialic acid from these receptors, thus allowing release of newly formed virus particles [4]. NAIs destroy the enzyme, causing aggregation of progeny virus, and decreasing spread to other, non-infected cells [5].

Four NAI have been developed to date: Oseltamivir, Zanamivir, Laninamivir and Peramivir. Of these, only Oseltamivir and Zanamivir are available for use in the USA.

Oseltamivir is the only oral NAI available in the USA. It is FDA approved for the prophylaxis and treatment of Influenza. Hayden et al. demonstrated that Oseltamivir had a protective efficacy of 74% and decreased the time to resolution of illness (from 96 h in the placebo group to 53 h in the treatment group) [6, 7]. Jefferson et al. conducted a review concluding that Oseltamivir decreased the duration of Influenza like illness (ILI) (hazard ratio 1.2, CI 1.06–1.35) [8], and decreased duration of viral shedding.

Zanamivir has extremely poor bioavailability and is administered by the inhalational route. Monto et al. demonstrated that Zanamivir decreased the duration of ILI by

1 day, and that initiation of treatment within 30 h of symptoms decreased duration of ILI by 1–1.5 days [9]. Exacerbation of bronchospasm in susceptible patients has been reported and thus limits widespread use of Zanamivir.

Both NAIs are FDA approved for use against Influenza A and B viruses, thus giving them a broader spectrum of activity than the Adamantanes, which selectively target influenza A viruses. The broader spectrum of antiviral activity was particularly relevant in the 2009 H1N1 influenza pandemic, when the CDC recommended Oseltamivir and Zanamivir as the drugs of choice for the treatment and prevention of pandemic influenza [10].

NAI Resistance

Oseltamivir

Prior studies have demonstrated Oseltamivir resistance in about 1.8% of adults receiving treatment, vs. up to 30% of patients developing Adamantane resistance while receiving those drugs [11]. In children treated with NAIs, resistance rates as high as 18% were observed, with the first resistant isolates identified on day 4 of treatment [12]. NAI resistance develops as a result of point mutations, leading to changes at or close to the site of the neuraminidase glycoprotein. In Influenza A (H3N2) viruses, the commonest mutation observed was an Arg292Lys mutation. By contrast, in Influenza A (H1N1) viruses, the commonest mutation recorded was the H274Y mutation. This mutation causes a 1,500 fold decrease in susceptibility to Oseltamivir, and a 500 fold decrease in susceptibility to Peramivir [13]. Animal models suggest that resistance makes the influenza virus less fit [14] (See Table 1).

During the 2007 to 2008 influenza season, the Influenza A (H1N1) virus predominated. Oseltamivir resistant Influenza was first detected in France and the United Kingdom [15]. By the end of this season, 22 of 30 countries in Europe reported Influenza A (H1N1) virus activity. Resistance ranged from 8.5% in Italy to 65% in Norway [15]. Regression analysis by Kramraz et al. demonstrated no statistical association between Oseltamivir use and subsequent emergence of resistant virus [16]. This same group however, found no difference in underlying characteristics, or clinical outcomes of the patients with Oseltamivir resistant Influenza. Of note, during this same time period, H1N1 retention of susceptibility to Zanamivir was observed. During this same season in the US, 12.3% of viruses tested were resistant to Oseltamivir [17]. Of the 264 viruses tested in early 2009, 98.5% were Oseltamivir resistant [17].

Zanamivir: Only one case of Zanamivir resistance has been reported in the literature [18]. An 18-month-old child with a bone marrow transplant was infected with Influenza B virus. On day 7, treatment with Zanamivir was initiated. She was maintained on treatment for 15 days, during which time she continued to shed the virus. The patient ultimately died of respiratory failure. The virus isolated on day 12 was 1,000-fold less sensitive to Zanamivir by inhibition assay than the one isolated on Day 1. In addition, this virus exhibited a mutation in the hemagglutanin glycoprotein that allowed for release of the virus from respiratory cells without need for activity of neuraminidase. This may have allowed the virus to circumvent the effect of Zanamivir.

Hurt et al. identified a novel mutation, Q136K, in 2.3% of Influenza A (H1N1) virus isolates obtained between 2006 and 2008. This mutation reduced susceptibility to Zanamivir and Peramivir, without affecting the efficacy of Oseltamivir (19). However, no other Zanamivir resistant mutants have been described in literature. As, worldwide, Oseltamivir is preferred over Zanamivir, likely due to ease of oral administration with the former, and possibility of bronchospasm with the latter, it is unknown if this mutation occurs under Zanamivir pressure.

While Oseltamivir resistance was an issue in the 2007 to 2008 and 2008 to 2009 Influenza seasons, viral isolates during the 2009 H1N1 pandemic Influenza were universally susceptible to the NAIs. The CDC recommended Oseltamivir and Zanamivir as drugs of choice for prevention and treatment of H1N1 pandemic Influenza A virus (10). However, in June 2009, an Oseltamivir resistant H1N1 pandemic Influenza A virus was isolated in Hong Kong [20] in a

Table 1 Common mutations leading to drug resistance

Drug	Common mutations leading to drug resistance
1. Adamantanes	
Amantadine	Multiple M2 gene point mutations including L26F, V27A, A30T, S31N, G34E, and V27A/S31N [44, 45]
Rimantadine	M2 gene point mutations at positions 27, 30, 31, 34, particularly S31N [46]
2. NAIs	
Oseltamivir	Influenza A (H3N2): Arg(292)Lys [R292K] mutation [13]
	Influenza A (H1N1): Hist(274)Tyr [H274Y] mutation [13] (N2 neuraminidase amino acid numbering)
Zanamivir	Influenza B (one documented case): Arg(152)Lys [R152K] [18]
	Influenza A (H1N1): Glu(136)Lys [Q136K] [19] Unknown clinical significance.
Peramivir	Hist(274)Tyr [H274Y] [13, 31] Possibly Hist(273)Tyr [H273Y] [47]
	Further research is needed to define resistance mutations.
Laninamivir	None published. Further research is needed to define resistance mutations

patient without prior exposure to the drug. Since then, multiple cases of Oseltamivir resistant viruses have been reported [21, 22]. Many occurred while patients were either on prophylaxis or treatment with Oseltamivir, and many were immunocompromised [22, 23]. The H274Y mutation was identified in resistant viral isolates. All isolates, however, retained their susceptibility to Zanamivir

Significance of Resistance

Thorlund et al. performed a review of literature in 2011, and identified 19 randomized controlled and observational trials reporting NAI resistance. Oseltamivir resistance was identified in 2% of patients with no resistance identified to Zanamivir. Patients infected with Oseltamivir resistant viruses were 4 times more likely to suffer from pneumonia than those infected with Oseltamivir sensitive viruses. No association with other clinical outcomes was identified, and no studies reporting association with Zanamivir resistance and clinical complications were identified [24].

During the 2008 influenza season, researchers in Norway assessed 272 viral samples for Oseltamivir resistance [25]. The investigators found no difference in viral shedding, primary symptoms, or overall complication and hospitalization rates between patients infected with Oseltamivir resistant versus vs. Oseltamivir sensitive virus. Although a higher number of patients infected with Oseltamivir resistant strains went on to develop pneumonia or sinusitis, this difference was not statistically significant.

In a prospective, observational study of hematopoietic stem cell transplant patients infected with H1N1 pandemic influenza 7 of 75 tested viral strains were Oseltamivir resistant [26]. Of these, six developed pneumonia ($p=0.005$), five needed mechanical ventilation ($p<0.001$) and three died from H1N1 or its complications ($p<0.01$). While these numbers were statistically significant, only a small proportion of samples were tested for resistance. As the number of cases reported with Oseltamivir resistant Influenza A (H1N1) is small, and involves immunocompromised hosts, it is difficult to extrapolate the results to a general population of H1N1 pandemic influenza patients.

Therefore, while several studies demonstrated little difference in outcomes in patients infected with Oseltamivir resistant H1N1 pandemic influenza, the findings of Thorlund et al. clearly demonstrate a fourfold increase in pneumonia complications for this subset of patients. If coupled with an emergent, more virulent influenza virus, the presence of Oseltamivir (or other NAI) resistance would significantly deplete the available arsenal of antiviral treatment options and heighten the risk of greater morbidity and mortality from H1N1 pandemic influenza.

Alternatives to Oseltamivir

Other NAIs

Zanamivir

Only one case of resistance has been reported in the literature [18]. This too was identified in a severely immunocompromised child. No further cases of resistance have been reported. This may be because the H274Y mutation does not affect Zanamivir sensitivity. Zanamivir use is not as wide spread as that of Oseltamivir. This is likely due to its route of administration, and its potential for causing bronchospasm. A concern is that increased use of this drug would induce the appearance of resistance

Laninamivir

This new NAI, administered by the inhalational route, is available for use in Japan. Mouse and ferret models demonstrated that a single dose of Laninamivir was superior to multiple doses of Oseltamivir or Zanamivir, and that this medication decreased viral titers in mice infected with an Oseltamivir resistant strain of Influenza A (H1N1) [27]. In a multi-center randomized clinical trial in Japan, 20 mg and 40 mg doses of Laninamivir were compared to Oseltamivir. In children infected with Oseltamivir resistant H1N1 Influenza A (with the H274Y mutation), Laninamivir decreased duration of illness by more than 60 h compared to Oseltamivir. In addition, on day 6, the proportion of children shedding the virus was significantly lower in the Laninamivir group [28•]. A similar effect was not seen in adults during this trial.

However, another publication reported that a single dose of Laninamivir was non-inferior to Oseltamivir in treating adults, with the higher dose of the drug associated with quicker alleviation of illness [29•]. Use of the higher dose of the drug corresponded with decreased viral shedding compared to Oseltamivir. The drug was well tolerated. Most of the H1N1 viruses in this study carried the H274Y mutation, and the Inhibition Concentration 50 (IC50) for Laninamivir was significantly lower than that of Oseltamivir. In patients infected with Influenza A H3N2, the higher dose of Laninamivir was non inferior to Oseltamivir in alleviation of illness, and viral shedding. Of note, all participants in this trial were otherwise healthy. Laninamivir as a 20 mg dose was approved for use in Japan in late 2010.

Could Laninamivir be an alternative to Oseltamivir in treatment of influenza? A single dose treatment makes this an ideal drug for use in pandemics, where large numbers of patients could be treated, by single drug administration, without concern for medication adherence. Greater research is needed into the safety and efficacy of this drug, particularly

in populations most at risk, such as the elderly and the immunocompromised.

Peramivir

Peramivir is an intravenous NAI, currently in Phase III trials. It was available by Emergency Use Authorization in the US for severe H1N1 pandemic influenza illness between October 2009 and June 23, 2010. In 31 Peramivir recipients, the drug was well tolerated with survival at 14, 28, and 56 days after initiation of treatment reported at 77%, 67%, and 59%, respectively [30]. Cross resistance with Oseltamivir with the H274Y remains a concern, as there was a 500-fold decrease in sensitivity to this drug in viruses with this mutation [13].

In 2010, Memoli et al. described two stem cell transplant patients [31•], both admitted with the H1N1 pandemic Influenza virus. The first patient was admitted with lower respiratory tract involvement, did not respond to 30 days of Oseltamivir, and continued to shed virus until day 44 after diagnosis. The second patient clinically worsened on Oseltamivir (24 days), and then received another 10 days of IV Peramivir, with continued viral shedding at days 30 and 44. This patient subsequently received a course of Zanamivir, responded clinically and then demonstrated negative nasopharyngeal washings on day 46. In both patients, virus collected on day 1 was susceptible, but during the course of treatment with Oseltamivir, and then Peramivir in the second case, isolates were discovered with the H274Y mutation. This lead to a >200 fold increase in IC50 to Oseltamivir, and a 50 fold increase in IC50 to Peramivir, while still retaining susceptibility to Zanamivir. Therefore, in these two immunocompromised hosts, mutations conferring resistance to both Oseltamivir and Peramivir were likely selected during treatment. This may significantly limit Peramivir use in the future.

Further studies on the efficacy and safety of this drug are needed.

Increased Vaccination

Molinari et al. estimated that the annual cost of influenza epidemics was about $87billion in the US [32]. Vaccine effectiveness (VE) is estimated at 41%, with a higher VE in adults >20 years of age, about 51% [33]. A Cochrane review in 2007, however, reported a vaccine efficacy as high as 80% when the vaccine matched the circulating strain [34]. At the same time, the CDC estimated that in January 2010, median coverage was only 38.3% for adults aged 18 to 49 years with high–risk conditions, 45.5% for adults aged 50 to 64 years, and 69.3% for adults aged 65 years and older [35]. It is believed that increased vaccination will decrease the number of individuals susceptible to Influenza, thus lowering the need for NAI use.

Prior to the appearance of H1N1 pandemic Influenza, the elderly, patients with chronic medical conditions, health care workers, pregnant women and children under the age of five were the primary targets for vaccination. However, during the pandemic, it was observed that up to 38% of hospitalized patients were in the 18 to 49 age group [36], with pregnant women accounting for 13% of mortality [37]. The latest CDC guidelines on vaccination for the 2011 to 2012 season recommend universal vaccination for all people over the age of 6 months [38]. In addition, the current vaccine will contain a strain derived from the 2009 H1N1pandemic Influenza virus.

Up to 70% of vaccine recipients are vaccinated in physician offices [39]. However, vaccination of adults in nontraditional settings, such as pharmacies and mass vaccination clinics, has been found to be cost-effective, or cost-saving [40]. Encouraging influenza vaccination in nontraditional settings may improve the low current rate of vaccination. Influenza vaccination is associated with a 60% reduction in days of illness, work days lost, days of presenteeism, and sick days in bed [41]. Therefore, in addition to decreasing the use of NAI, increased influenza vaccination would decrease morbidity.

Combination Therapy

In an effort to limit antiviral resistance, some authorities have suggested combination therapy as a treatment option for Influenza. Mouse models suggest that combination therapy with Oseltamivir and Amantidine, in mice infected with Amantadine sensitive H5N1 virus, provide greater protection against lethal infection than each used alone (60% and 90%, respectively). This advantage was not seen in H5N1 viruses resistant to Amantadine. Interestingly, no Neuraminidase, Hemagglutinin or M2 mutations developed during the study [42].

A randomized, controlled, double blind trial comparing Zanamivir and Rimantidine against Rimantidine alone demonstrated a statistically significant decrease in symptoms (cough) by day 3 of treatment (p-0.01) [43]. In addition, while 2 patients developed Rimantidine resistance while on monotherapy, neither Rimantidine nor Zanamivir resistance developed in the combination group. Due to small sample size and premature study termination, the study lacked statistical power, yet demonstrated a non-statistically significant trend towards decreased viral shedding.

More clinical trials are needed to determine if combination therapy is more effective than single drug therapy, and whether it would decrease emergence of antiviral resistant influenza virus.

Conclusions

With the rapid development of Adamantane resistance, NAIs remain the main antivirals used in the treatment of Influenza. Of these, Oseltamivir is most widely used. Increasing Oseltamivir resistance may portend a bleak future in our battle against the H1N1 influenza virus. The current literature reports a fourfold increase in rates of pneumonia in patients infected with Oseltamivir resistant viruses, and higher rates of mechanical ventilation and death in immunocompromised patients. Alternatives include Zanamivir, and newer NAIs such as Peramivir and Laninamivir. However, development of antiviral resistance with increased use of these medications seems likely. Increasing rates of vaccination may help decrease the population at risk for developing influenza. Further studies of both new antiviral drugs and combination antiviral therapy are needed to best determine treatment practices for influenza.

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