Treatment and prophylaxis of influenza and the problem of resistance to neuraminidase inhibitors

Leczenie i profilaktyka grypy a problem oporności na inhibitory neuraminidazy

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Summary

Influenza virus neuraminidase inhibitors (NAIs), including oseltamivir, zanamivir and peramivir, are important antivirals for the treatment and prophylaxis of influenza. Increasing use of NAIs brings into focus the risk of drug resistance. The problem of resistance is of high clinical and epidemiological importance. There are generally three levels of antiviral resistance according to the way that resistance can be detected or inferred: genotypic, phenotypic and clinical resistance. Recently the problem of resistance to NAIs, although still rare (<2% of influenza isolates), has been rising. It should be underlined that NAI resistance in influenza viruses is relative, and despite its presence patients with resistant viruses may still benefit from receiving NAIs. The clinical resistance and the response to treatment with antivirals remain the most important proof of antiviral effectiveness. Currently, there has not been observed cross-resistance between oseltamivir and zanamivir, which may be a consequence of the number of given doses, differences in drug structure and duration of the drug concentrations in the site of infection. Early treatment with appropriate doses of NAI is necessary to minimize the likelihood of a resistant virus arising.

Key words: influenza • resistance • neuraminidase inhibitors

INTRODUCTION

Neuraminidase inhibitors (NAIs), including oseltamivir, zanamivir, peramivir and laninamivir, are an important class of antivirals for the treatment and prophylaxis of influenza. In contrast to the older class of antivirals – the adamantanes (amantadine and rimantadine) – NAIs are effective against both influenza virus type A and B; they are associated with fewer side effects and a better profile concerning drug resistance [2,5,25]. Adamantane-resistant isolates of influenza A viruses are generally stable, can be transmitted to susceptible contacts and can be shed for prolonged periods in immunocompromised patients taking the drug. This potential for the development of resistance especially limits the use of the adamantanes for the treatment and prophylaxis of seasonal influenza. These drugs should not be used during an influenza epidemic (90% of viruses are resistant); nor are they recommended for pandemic influenza caused by influenza virus A (H1N1)pdm09 [4]. Adamantanes are not recommended for the treatment and prophylaxis of avian influenza – also because of the problem of resistance [3,4].

Because of the high resistance of influenza virus to adamantanes (99%), the newer group of antivirals – neuraminidase inhibitors – seems to be the best choice for treatment and prophylaxis for seasonal influenza, avian influenza and pandemic influenza [3].

The rational use of NAIs is necessary to preserve their potential power in fighting against influenza, which is why the problem of influenza viruses resistant to NAIs should be considered as a current medical problem of high impact.

MECHANISM OF ACTION OF NAIs

The neuraminidase inhibitors interfere with the release of progeny influenza virus from infected host cells. All influenza viruses bear two surface glycoproteins: a hemagglutinin and a neuraminidase. The neuraminidase, the target molecule of the neuraminidase inhibitor compounds, cleaves the cellular-receptor sialic acid residues to which the newly formed particles are attached. Without neuraminidase, infection would be limited to one round of replication. The mechanism of action of NAI explains the necessity of the early use of these drugs: ideally 36-48 hours after the onset of clinical symptoms of the disease [2,5,21].

CURRENTLY USED NAIs

There are two neuraminidase inhibitors currently registered in Poland: oseltamivir (Tamiflu, Roche) and zanamivir (Relenza, GSK). Only oseltamivir is available on the market [2,21]. A third NAI, peramivir, is available in intravenous form and is reserved for critically ill patients. Three influenza antiviral medications approved by the U.S. Food and Drug Administration (FDA) were recommended for use in the United States during the 2014-2015 influenza season: oral oseltamivir (Tamiflu), inhaled zanamivir (Relenza), and intravenous peramivir (Rapivab) [3].

INDICATIONS FOR NAIs

Seasonal influenza

NAIs are recommended for treatment and prophylaxis of seasonal influenza. Recommended duration and doses of the NAIs are presented in table 1.

TREATMENT

Early initiation of treatment enables the reduction of duration of symptoms (mostly fever); treated patients have a lower frequency of secondary complications (e.g., otitis media in young children, pneumonia, and respiratory failure) [3,6]. Early treatment of hospitalized patients can reduce the risk of death; in hospitalized children, early antiviral treatment has been shown to shorten the duration of hospitalization [19,30]. The clinical benefit is greatest when antiviral treatment is administered early, especially within 48 hours of influenza illness onset [17,33]. Antiviral treatment is recommended as early as possible for any patient with confirmed or suspected influenza who: is hospitalized; has severe, complicated, or progressive illness; or is at higher risk for influenza complications (listed in table 2) [3].

The clinical judgment based on the patient's disease severity and progression, age, underlying medical conditions, likelihood of influenza, and time since onset of symptoms is important when making antiviral treatment decisions for high-risk outpatients. Antiviral treatment should be started as soon as possible after illness onset, ideally within 48 hours of symptom onset. However, antiviral treatment might have some benefits in patients with severe, complicated or progressive illness, even when started after 48 hours of the illness onset [14,39]. Antiviral treatment of pregnant women (of any trimester) with influenza A (2009 H1N1) virus infection has been shown to be most beneficial in preventing respiratory failure and death when started within 3 days of illness onset, but still provided benefit when started 3–4 days after onset compared to 5 or more days [27]. Antiviral treatment also can be considered for any previously healthy, symptomatic outpatient not at high risk with confirmed or suspected influenza on the basis of clinical judgment, if treatment can be initiated within 48 hours of the illness onset [3]. The recommended treatment course for uncomplicated influenza is two doses per day of oral oseltamivir or inhaled zanamivir for 5 days, or one dose of intravenous peramivir for 1 day. Oral oseltamivir is preferred for treatment of pregnant women [24]. Pregnant women are recommended to receive the same antiviral dosing as non-pregnant persons. For hospitalized patients and patients with severe or complicated illness, treatment with oral or enterically administered oseltamivir is preferred.

Inhaled zanamivir is not recommended because of the lack of data for use in patients with severe influenza disease. There are also insufficient data regarding efficacy of intravenous peramivir for hospitalized patients [3].

The optimal duration and dose are uncertain for severe or complicated influenza. The clinical judgment should be the guide regarding the need to extend treatment regimens longer than 5 days for patients whose illness is prolonged. Longer treatment regimens might be necessary in immunosuppressed persons who may have prolonged influenza viral replication. Such patients are at risk of developing an antiviral-resistant virus [3]. A higher dose of oral or enterically administered oseltamivir has been recommended by some experts (e.g., 150 mg twice daily in adults with normal renal function) for treatment of influenza in immunocompromised patients and in severely ill hospitalized patients. One must remember that if a hospitalized patient treated with NAIs manifests progressive lower respiratory symptoms, a resistant virus should be considered [3].

Chemoprophylaxis

Without any doubt, the annual influenza vaccination is the best way to prevent influenza [2]. However, antivirals are useful adjuncts to influenza vaccination, but they are effective only in 70-90% of cases for preventing the disease [10,11,21]. Widespread or routine use of NAIs for chemoprophylaxis is not recommended as it could raise the risk of resistance. NAIs can be considered for chemoprophylaxis in some situations [3]:

• prevention of influenza in persons at high risk of influenza complications during the first two weeks following vaccination after exposure to an infectious person;
• prevention for people with severe immune deficiencies or others who might not respond to influenza vaccination, such as persons receiving immunosuppressive medications, after exposure to an infectious person;
• prevention for people at high risk for complications from influenza who cannot receive influenza vaccine due to a contraindication after exposure to an infectious person;
• prevention of influenza among residents of institutions, such as long-term care facilities, during influenza outbreaks in the institution.

To be effective as chemoprophylaxis, the antiviral medication must be taken each day for the duration of potential exposure to a person with influenza and continued for 7 days after the last known exposure. For persons taking antiviral chemoprophylaxis after inactivated influenza vaccination, the recommended duration is until immunity after vaccination develops (antibody development after vaccination takes about two weeks in adults and can take longer in children depending on age and vaccination history) [3,10,11]. Prophylaxis with NAIs generally is not recommended if more than 48 hours have elapsed since the first exposure to an infectious person.

The use of antiviral chemoprophylaxis to control outbreaks among high risk persons in institutional settings is also recommended (regardless of their vaccination status). For newly vaccinated staff, antiviral chemoprophylaxis can be administered for up to two weeks (the time needed for antibody development) following influenza vaccination. Chemoprophylaxis may also be considered for all employees, regardless of their influenza vaccination status, if the outbreak is caused by a strain of influenza virus that is not well matched by the vaccine. The antiviral chemoprophylaxis should be administered for a minimum of two weeks, and continued for at least seven days after the last known case was identified [3,10,11].

AVIAN ORIGIN INFLUENZA

As of 31 March 2015, a total of 826 cases of avian influenza A (H5N1) had been reported, with 440 deaths (mortality rate 53%) [34,35,36,37]. Oseltamivir is a drug of choice for treatment of influenza caused by A (H5N1) virus. To date there are no patients treated with zanamivir, although it is active against H5N1 virus in an animal model and in vitro [26,32,40].

Oseltamivir and zanamivir may be used in prophylaxis of avian influenza caused by influenza A (H5N1) virus. Chemoprophylaxis against H5N1 infection should not be routinely offered to low-risk groups, including health-care workers without direct exposure to A (H5N1) infection or healthcare or poultry workers who used appropriate protective equipment during potential exposure [1,13]. The choice of antiviral drug should be based on sensitivity testing when possible (strongly indicated). Zanamivir may be considered a suitable alternative to oseltamivir exposure [1,26].

The novel avian A (H7N9) influenza virus has caused more than 130 human infections with 43 deaths (as of September, 2013) in China [36]. On 10 April 2015, the National Health and Family Planning Commission of China notified the WHO of 20 additional laboratory-confirmed cases of human infection with avian influenza A (H7N9) virus, including 4 deaths [34]. The majority (90%) reported exposure to live poultry. One case concerned a health care worker, who also had poultry exposure. No clusters were reported. Neuraminidase inhibitors are the only licensed antiviral medications available to treat avian influenza A(H7N9) virus infections in humans [34]. The WHO continues to recommend antiviral treatment with a neuraminidase inhibitor as soon as possible for patients with suspected or confirmed H7N9 virus infection; antiviral treatment should not be delayed while H7N9 laboratory test results are pending. Persons who have had unprotected close contact with a patient with confirmed H7N9 virus infection or exposure to poultry, a live poultry market or environments contaminated by H7N9 virus should be monitored for 7 days after the last known exposure. If fever or any respiratory symptom develops, empiric antiviral treatment should be started immediately, and respiratory specimens should be collected for

H7N9 virus testing [34]. Empiric early antiviral treatment with a neuraminidase inhibitor for 5 days is recommended. A clinical decision should be made about whether to extend the duration of the antiviral treatment. The WHO does not recommend routine post-exposure antiviral chemoprophylaxis for H7N9 virus. However, for some asymptomatic persons in whom substantial unprotected or prolonged exposure to a patient with H7N9 infection has occurred, initiation of empiric post-exposure antiviral treatment (e.g. oseltamivir 75 mg orally twice daily for 5 days), on the presumption that influenza virus infection has occurred, may be considered. This is likely to be limited to healthcare or other settings involving substantial exposure of those at higher risk for complications from influenza virus infection, including, but not limited to, patients with severe immunosuppression, neonates and infants, pregnant and early post partum women, elderly adults, persons with chronic co-morbidities and, other highly vulnerable patients; or unprotected healthcare workers, especially those involved in aerosol-generating procedures [34].

RESISTANCE TO NAIS – GENERAL CONSIDERATIONS

A key advantage of neuraminidase inhibitors, and a major difference from the adamantanes, is that the development of resistance is very rare (<1%) [15,18]. The problem of resistance is of a high importance – hence the creation of the global Neuraminidase Inhibitor Susceptibility Network (NISN), which coordinates the analysis of clinical isolates collected through the WHO surveillance network. Surveillance of antiviral susceptibility of influenza viruses circulating in Europe was established in 2004 though the European Union-funded European Surveillance Network for Vigilance against Viral Resistance (VIRGIL), in collaboration with the European Influenza Surveillance Scheme (EISS), the WHO and national influenza centers [18].

There are generally three levels of antiviral resistance according to the way that resistance can be detected or inferred: genotypic resistance, phenotypic resistance, and clinical resistance. Resistance to neuraminidase inhibitors may be due to mutations in hemagglutinin, which often confers resistance to both zanamivir and oseltamivir, while mutation in neuraminidase may render oseltamivir ineffective but retains susceptibility to zanamivir [8].

NA mutations selected from in vitro and in vivo experiments are limited to several conserved or semiconserved residues: R292K and E119G/A/D/V in N9 and N2 subtypes, H274Y in the N1 subtypes; and E119G, D198N and R152K in influenza B virus NA [15,16]. Influenza virus variants with the N294S mutation in NA were recently isolated after oseltamivir treatment from patients treated either for H3N2 or H5N1 influenza viruses [15,16].

What should also be underlined, oseltamivir resistance in influenza viruses is relative, and despite its presence patients with oseltamivir-resistant viruses may still benefit from receiving oseltamivir [20]. The clinical course

for patients with resistant viruses and those treated with antivirals is not any different from patients carrying fully sensitive strains. The clinical resistance and the response to treatment with antivirals (the clinical response) remain the most important proof of the antiviral effectiveness [3].

Patients receiving antiviral medications who do not respond to treatment might have an infection with an antiviral-resistant influenza virus. Oseltamivir resistance, sometimes within 1 week of treatment initiation, has been reported particularly among immunocompromised patients with 2009 H1N1 virus infection who were receiving treatment with oseltamivir [5]. Infection-control measures are especially important for patients who are immunocompromised to reduce the risk for transmission of oseltamivir-resistant viruses. Zanamivir is the treatment of choice for all patients where oseltamivir resistance is demonstrated or highly suspected. Intravenous zanamivir may be considered where available [3,4,5].

RESISTANCE TO NAIS BEFORE 2009

Viral resistance to oseltamivir may develop by alteration of the amino acid composition of neuraminidase or by alteration in the affinity of hemagglutinin to the receptors on the cell surface. In addition, according to a recent study, a few influenza strains may completely lack neuraminidase activity, which may also result in viral resistance to neuraminidase inhibitor. Resistant strains have been generated in vitro, and such strains have also been found in a small proportion of patients during or after treatment with oseltamivir. Oseltamivir-resistant strains have also been detected in individuals not exposed to oseltamivir. Mutations in the viral neuraminidase gene can be generated in vitro by repeated passages in the presence of low concentrations of oseltamivir [12].

In patients treated with oseltamivir the frequency of resistant viruses was estimated as 1-2% in adults and 5-6% in children [4,8,18]. The clinical course of influenza in oseltamivir-treated patients, from whom the resistant viruses were isolated, appeared to be similar to that with wild-type virus [4,20]. Predominant mutations of NA were Arg292Lys and Glu119Val in the H3N2 virus. An oseltamivir-resistant virus was isolated from 16.3% of Japanese children treated with oseltamivir for influenza A (H3N2) infections (2004) [29,30]. All were due to A His274Tyr mutations in H1N1 viruses. The authors attributed the higher incidence of oseltamivir resistance to high viral titers and protracted viral shedding, more rigorous detection methods or relatively lower oseltamivir dosage (4 mg/kg regardless of the body mass). The small number of children in this study (43) is also considered a limitation of its results; however, it is underlined that the rate of oseltamivir resistance in children should be of concern because children are the most important source of the disease and play an important role in transmission of the disease [30]. The Japanese situation should also be treated as a warning of the overuse of NAIs.

The significance of the resistant strains observed in individuals who were not exposed to oseltamivir is unclear at the present. Such a situation was described in the 2007/2008 seasons in Europe. Results from analysis of the early winter (November 2007 – January 2008) A (H1N1) virus isolates revealed a significant proportion, approximately 14%, of European strains resistant to oseltamivir, but retaining sensitivity to zanamivir (and the adamantanes [12]. A total of 437 influenza A (H1N1) viruses were tested using measurement of NA enzyme activity in the presence of oseltamivir to determine the drug ensitivity (IC_{50}) of the viral enzyme in conjunction with sequence analysis of the viral neuraminidase gene. Oseltamivir resistance viruses have been detected in 9 countries (Denmark, Finland, France, Germany, Netherlands, Norway, Portugal, Sweden and the UK); in particular, in Norway, France, Germany and the UK, 70%, 17%, 7% and 5%, respectively, carry the same mutation causing the substitution of histidine by tyrosine at residue 274 (H274Y) of the neuraminidase, which is known to confer a high level of resistance to oseltamivir [12]. Viruses bearing this mutation, when tested in enzyme assays, showed a reduction of approximately 400-fold in susceptibility to oseltamivir (IC_{50} values increased from 1 nM to more than 400 nM). All these viruses remain sensitive to the other anti-neuraminidase drug zanamivir and to the anti-M2 drugs amantadine and rimantadine. The resistant (H274Y) viruses have been isolated from both adults and children, ranging from 1 month to 61 years in age, with the majority of viruses being isolated from adults. There was no information that these viruses were isolated from patients who had been either treated with oseltamivir or in close contact with another individual treated with this drug [20].

These findings indicate the necessity for careful virological and epidemiological surveillance concerning oseltamivir resistance, but it is also agreed that there is currently insufficient evidence for the authorities to consider changes to clinical guidelines [20].

It was also underlined that oseltamivir was not frequently used in Europe, although better data need to be acquired in this field, which is why the described resistance was related to antiviral medication usage in individual patients. There is also currently no evidence that the mutated H1N1 viruses are more virulent than other strains of seasonal influenza (all the Norwegian patients had typical influenza illness symptoms).

The susceptibility of above-mentioned isolates from 1999-2002, post-licensure of the neuraminidases, was monitored and only two isolates (0.1%) had reduced susceptibility to zanamivir and possible resistance-associated mutations. The clinical use of zanamivir is still limited but, so far, zanamivir-resistant viruses have not been isolated from immunocompetent individuals who have received zanamivir [4]. One resistant virus was isolated from an immunocompromised child after bone-marrow transplantation infected with type B influenza virus [9].

The mutant showed a small decrease in sensitivity to zanamivir in infected mice but there was no detectable resistance to zanamivir in ferrets. Immunocompromised patients have difficulties with cleaning virus and this appears to promote selection of a drug-resistant virus.

As with oseltamivir, mutations that confer resistance to zanamivir may also reduce the virulence of the virus. To date, influenza virus strains which are resistant to oseltamivir remain susceptible to zanamivir – *in vitro.* The lack of cross-resistance between oseltamivir and zanamivir may be explained by the longer use of oseltamivir and a limited number of zanamivir dosages, but there are other hypotheses to describe the lack of cross-resistance [9,29].

It is possible that differences in chemical structure and binding to the NA catalytic site result in different drug resistance profiles. This has been attributed to how closely the compounds mimic the transition state analog for NA. Hence zanamivir, which closely resembles the natural substrate for NA, has a low resistance index [9,29,31].

Although both drugs, zanamivir and oseltamivir, are based on the transition state analog of sialic acid, zanamivir has a single substitution of a guanidine group at the 4' position on the sugar ring, whereas oseltamivir has an amino group at the 4 position and, more importantly, a bulky hydrophobic pentyl ether group replacing the glycerol side chains at the 6' position. Reorientation of E 276 in the active site is required to create a hydrophobic pocket necessary to accommodate the pentyl ether group. Mutations that prevent this reorientation occurring lead to high levels of specific oseltamivir resistance (H 274Y, R292K), while for zanamivir this reorientation is not required [38].

It is also considered that differences in the mode of delivery and pharmacokinetics of zanamivir have implications for drug resistance. Differences in concentrations of NAIs at the site of viral replication could contribute to differences not only in efficacy but also in the risk of emergence of NAI-resistant viral strains. Low drug concentrations, which only partly block viral replication, could enhance the risk by providing an environment for a drug-resistant virus to emerge [38].

RESISTANCE TO NAIs AFTER 2009

In the winter of 2007-2008, an oseltamivir-resistant seasonal influenza A(H1N1) strain with an H274Y mutation emerged in the northern hemisphere and spread rapidly around the world. In contrast to earlier evidence of such resistant viruses being unfit, this mutant virus remained fully transmissible and pathogenic and became the major seasonal A(H1N1) virus globally within a year [15,16]. This resistant A(H1N1) virus was displaced by the sensitive A(H1N1)pdm09 virus. Approximately 0.5-1.0% of community A(H1N1)pdm09 isolates are currently resistant to oseltamivir. It is now apparent that variation in non-active site amino acids can affect the fitness of

Table 1. Recommended dosage and duration of NAIs for treatment or chemoprophylaxis of influenza [3]

Medication	Use, duration, dose	Recommended for	Adverse events
Oseltamivir (Tamiflu)	Treatment: 5 days Children < 1 year 3 mg/kg/dose twice daily > 1 year <15 kg: 2x 30 mg 15-23 kg: 2x 45 mg 23-40 kg: 2x 60 mg >40 kg: 2x 75 mg Adults 2x 75 mg	Any age1	Adverse events: nausea, vomiting. Postmarketing reports of serious skin reactions and sporadic, transient neuropsychiatric events (self-injury or delirium; mainly reported among Japanese adolescents and adults).
	Chemoprophylaxis: 7 days Children for infants < 3 months old, the use of oseltamivir for chemoprophylaxis is not recommended unless the situation is judged critical due to limited data in this age group. 3-12 months 1x 3 mg/kg/dose > 1 year <15 kg: 1x 30 mg 15-23 kg, 1x 45 mg 23-40 kg, 1x 60 mg >40 kg, 1x 75 mg Adults 1x 75 mg	>3 months	
Zanamivir (Relenza)	Treatment: 5 days 2x10 mg	>7 years	Allergic reactions: oropharyngeal or facial edema. Adverse events: diarrhea, nausea, sinusitis, nasal signs and symptoms, bronchitis, cough, headache, dizziness, and ear, nose and throat infections.
	Chemoprophylaxis: 7 days 1x 10 mg	> 5 years	
Peramivir (Rapivab)	Treatment: 1 day One 600 mg dose, via intravenous infusion for 15-30 minutes	> 18 years	Adverse events: diarrhea. Postmarketing reports of serious skin reactions and sporadic, transient neuropsychiatric events (self-injury or delirium; mainly reported among Japanese adolescents and adults).
	Chemoprophylaxis – not recommended		

the enzyme and compensate for mutations that confer high-level oseltamivir resistance resulting in a minimal impact on enzyme function [15,16].

The development of resistance to oseltamivir during treatment was more common among seasonal influenza A (H1N1) virus infections (27%) compared with seasonal influenza A (H3N2) (3%) or B (0%) viruses [4,5]. Sporadic cases of resistance to oseltamivir have been observed among persons with 2009 H1N1 virus infection (e.g., immunosuppressed patients with prolonged viral replication during oseltamivir treatment and persons who developed the illness while receiving oseltamivir chemoprophylaxis) [4,5]. Emergence of oseltamivir-resistant 2009 H1N1 virus

Table 2. Persons at higher risk for influenza complications who are recommended for antiviral treatment [3]

children aged younger than 2 years

adults aged 65 years and older

persons with chronic pulmonary (including asthma), cardiovascular (except hypertension alone), renal, hepatic, hematological (including sickle cell disease), and metabolic disorders (including diabetes mellitus), or neurologic and neurodevelopment conditions (including disorders of the brain, spinal cord, peripheral nerve, and muscle, such as cerebral palsy, epilepsy [seizure disorders], stroke, intellectual disability [mental retardation], moderate to severe developmental delay, muscular dystrophy, or spinal cord injury)

persons with immunosuppression, including that caused by medications or by HIV infection;

women who are pregnant or postpartum (within 2 weeks after delivery)

persons aged younger than 19 years who are receiving long-term aspirin therapy

persons who are morbidly obese (i.e., body mass index is equal to or greater than 40); and

residents of nursing homes and other chronic care facilities

strains within 48 hours after initiation of treatment has been reported [4,5]. Transmission of oseltamivir-resistant influenza B virus strains or 2009 H1N1 virus strains acquired from persons treated with oseltamivir is rare but has been documented [4,5].

Rare cases of infection with the 2009 H1N1 virus resistant or with reduced susceptibility to multiple neuraminidase inhibitors in severely immunosuppressed pediatric patients with prolonged viral replication have been reported [4,5].

A recently published meta-analysis of 15 studies yielded a pooled incidence rate for oseltamivir resistance of 2.6% [29]. The incidence rate for all zanamivir resistance studies was 0%. Only one study measured incidence of antiviral resistance among subjects given peramivir, and it was reported to be 0%. Subgroup analyses detected higher incidence rates among influenza A patients, especially for H1N1 subtype influenza. Considerable heterogeneity between studies precluded definite inferences about subgroup results for immunocompromised patients, in-patients, and children. A meta-analysis of 4 studies reporting an association between oseltamivir resistance and pneumonia yielded a statistically significant risk ratio of 4.2. Oseltamivir resistance was not statistically significantly associated with other clinical complications and symptoms [29]. According to Okomo et al., influenza A(H1N1)pdm09 viruses were sensitive to all NAIs, except for two (0.6%) with H275Y (N1 numbering; H274Y in N2 numbering) substitution, which exhibited elevated IC_{50}s for oseltamivir and peramivir, and a third with previously unreported N325K substitution, exhibiting reduced susceptibility to oseltamivir. Influenza A(H3N2) viruses were sensitive to all NAIs [23]. Influenza B viruses were sensitive to all NAIs, except two (0.6%) with H273Y (N1 numbering; H274Y in N2 numbering) substitution, exhibiting reduced susceptibility to oseltamivir and peramivir, and one with previously unreported G140R and N144K substitutions, exhibiting reduced susceptibility to oseltamivir, zanamivir, and peramivir. All influenza A and B viruses were sensitive to laninamivir. It is unknown whe-

ther substitutions N325K, G140R, and N144K were present in the virus prior to culturing because clinical specimens were unavailable for testing [23]. Recently published data do not support a possible transfer of oseltamivir resistance mutations from avian to human influenza A virus strains [22]. It has also been found that influenza viruses with B/Yamagata and B/Victoria-like neuraminidases are differentially affected by mutations that may alter the antiviral susceptibility. Framework residue mutations E117A and E117G confer highly reduced inhibition to three of the four NAIs, but substantially reduced neuraminidase activity, whereas other framework mutations retained a greater level of NA activity. Mutations E105K, P139S and G140R of the monomeric interface were also found to cause highly reduced inhibition, but, interestingly, their effect was substantially greater in a B/Victoria-like neuraminidase than in a B/Yamagata-like neuraminidase, with some susceptibility values being up to 1000-fold different between lineages [7].

According to the global update on the susceptibility of human influenza viruses to neuraminidase inhibitors in 2013-2014, approximately 2% of influenza strains showed highly reduced inhibition (HRI) against at least one of four NAIs, commonly oseltamivir, while 0.3% showed reduced inhibition (RI). Those showing HRI were A(H1N1)pdm09 with NA H275Y A(H3N2) with NA E119V, B/Victoria-lineage with NA E117G and B/Yamagata-lineage with NA H273Y (n=1); amino acid position numbering is A subtype and B type specific. Although approximately 98% of circulating viruses tested during the 2013-2014 period were sensitive to all four NAIs, a large community cluster of A(H1N1)pdm09 viruses with the NA H275Y substitution from patients with no previous exposure to antivirals was detected in Hokkaido, Japan. Significant numbers of A(H1N1)pdm09 NA H275Y viruses were also detected in China and the United States: phylogenetic analyses showed that the Chinese viruses were similar to those from Japan, while the United States viruses clustered separately from those of the Hokkaido outbreak, indicative of multiple resistance emergence events. Consequently, global surveillance of influenza

antiviral susceptibility should be continued from a public health perspective [28].

Conclusions

1. Neuraminidase inhibitors are potent antivirals for prophylaxis and treatment of seasonal influenza, avian influenza and a future influenza pandemic.

2. Global collaboration and phenotypic and genotypic testing of drug sensitivity of circulating influenza viruses for NA inhibitor sensitivity are critical.

3. In order to limit the risk of spreading resistant strains of influenza viruses to neuraminidase inhibitors, it is necessary to carefully diagnoses and treat all cases of seasonal and avian influenza.

References

[1] Bridges C.B., Katz J.M., Seto W.H., Chan P.K., Tsang D., Ho W., Mak K.H., Lim W., Tam J.S., Clarke M., Williams S.G., Mounts A.W., Bresee J.S., Conn L.A., Rowe T. et al.: Risk of influenza A (H5N1) infection among health care workers exposed to patients with influenza A (H5N1), Hong Kong. J. Infect. Dis., 2000; 181: 344-348

[2] Brydak L.: Influenza. Myth or a real threat? Termedia Ed., Warsaw 2008: 23-87

[3] Center for Disease Prevention and Control (CDC). Influenza antiviral medications: summary for clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm (21 April 2015)

[4] Center for Disease Prevention and Control (CDC). Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices-United States, 2013-14. http://www.cdc.gov/flu/professionals/acip/index.htm. (24 April 2015)

[5] Center for Disease Prevention and Control (CDC). Recommended dosage and duration of influenza antiviral medications for treatment or chemoprophylaxis. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm#dosage (25 April 2015)

[6] European Medicines Agency (EMA). Updated review of influenza antiviral medicinal products for potential use during pandemic by the Committee for Medical Products for Human Use (CHMP) of the European Medicine Agency. ww.ema.europa.eu/ema/index.jsp (12 April 2014)

[7] Farrukee R., Leang S.K., Butler J., Lee R.T., Maurer-Stroh S., Tilmanis D., Sullivan S., Mosse J., Barr I.G., Hurt A.C.: Influenza viruses with B/Yamagata- and B/Victoria-like neuraminidases are differentially affected by mutations that alter antiviral susceptibility. J. Antimicrob. Chemother., 2015; 70: 2004-2012

[8] Gubareva L.V.: Molecular mechanisms of influenza viruses resistance to neuraminidase inhibitors. Virus Res., 2004; 103: 199-203

[9] Gubareva L.V., Matrosovich M.N., Brenner M.K., Bethell R.C., Webster R.G.: Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. J. Infect. Dis., 1998; 178: 1257-1262

[10] Hayden F.G., Gubereva L.V., Monto A.S., Klein T.C., Elliot M.J., Hammond J.M., Sharp S.J., Ossi M.J.; Zanamivir Family Study Group: Inhaled zanamivir for the prevention of influenza in families. N. Engl. J. Med., 2000; 343: 1282-1289

[11] Hayden F.G., Belshe R., Villanueva C., Lanno R., Hughes C., Small I., Dutkowski R., Ward P., Carr J.: Management of influenza in households: a prospective, randomized comparison of oseltamivir treatment with or without postexposure prophylaxis. J. Infect. Dis., 2004; 189: 440-449

[12] Lackenby A., Hungnes O., Dudman S.G., Meijer A., Paget W.J., Hay A.J., Zambon M.C.: Emergence of resistance to oseltamivir among influenza A(H1N1) viruses in Europe. Euro. Surveill., 2008; 13: 1-2

[13] Le Q.M., Kiso M., Someya K., Sakai Y.T., Nguyen T.H., Nguyen K.H., Pham N.D., Ngyen H.H., Yamada S., Muramoto Y., Horimoto T.,

Takada A., Goto H., Suzuki T., Suzuki Y., Kawaoka Y.: Avian flu: isolation of drug resistant H5N1 virus. Nature, 2005; 437: 1108

[14] Louie J.K., Yang S., Acosta M., Yen C., Samuel M.C., Schechter R., Guevara H., Uyeki T.M. Treatment with neuraminidase inhibitors for critically ill patients with influenza A (H1N1)pdm09. Clin. Infect. Dis., 2012; 55: 1198-1204

[15] McKimm-Breschkin J.L.: Resistance of influenza viruses to neuraminidase inhibitors. Antiviral Res., 2000; 47: 1-17

[16] McKimm-Breschkin J.L., Williams J., Barrett S., Jachno K., McDonald M., Mohr P.G., Saito T., Tashiro M.: Reduced susceptibility to all neuraminidase inhibitors of influenza H1N1 viruses with haemagglutinin mutations and mutations in non-conserved residues of the neuraminidase. J. Antimicrob. Chemother., 2013; 68: 2210-2221

[17] Monto A.S., Fleming D.M., Henry D., de Groot R., Makela M., Klein T., Elliott M., Keene O.N., Man C.Y.: Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza A and B virus infection. J. Infect. Dis., 1999; 180: 254-261

[18] Monto A.S., McKimm-Breschkin J.L., Macken C., Hampson A.W., Hay A., Klimov A., Tashiro M., Webster R.G., Aymard M., Hayden F.G., Zambon M.: Detection of influenza viruses resistant to neuraminidase inhibitors in global surveillance during the first 3 years of their use. Antimicrob. Agents Chemother., 2006; 50: 2395-2402

[19] Nicholson K.G., Aoki F.Y., Osterhaus A.D., Trottier S., Carewicz O., Mercier C.H., Rode A., Kinnersley N., Ward P.: Efficacy and safety of oseltamivir in treatment of acute influenza: a randomized controlled trial. Lancet, 2000; 355: 1845-1850

[20] Nicoll A., Ciancio B., Kramarz P., Influenza Project Team: Observed oseltamivir resistance in seasonal influenza viruses in Europe interpretation and potential implications. Euro. Surveill., 2008; 13: 1-2

[21] Nitsch-Osuch A., Brydak L.B., Wardyn A.K.: Neuraminidase inhibitors in prophylaxis and treatment of influenza. Pol. Merkur. Lekarski, 2008; 25: 67-73

[22] Norberg P., Lindh M., Olofsson S.: Published sequences do not support transfer of oseltamivir resistance mutations from avian to human influenza A virus strains. BMC Infect. Dis., 2015; 15: 162

[23] Okomo-Adhiambo M., Sleeman K., Lysen C., Nguyen X.T., Xu X., Li Y., Klimov A.I., Gubareva L.V.: Neuraminidase inhibitor susceptibility surveillance of influenza viruses circulating worldwide during 2011 Southern Hemisphere season. Influenza Other Respir. Viruses, 2013; 7: 645-658

[24] Rasmussen S.A,. Kissin D.M., Yeung L.F., MacFarlane K., Chu S.Y., Turcios-Ruiz R.M., Mitchell E.W., Williams J., Fry A.M., Hageman J., Uyeki T.M., Jamieson D.J., Pandemic Influenza and Pregnancy Working Group: Preparing for influenza after 2009 H1N1: special considerations for pregnant women and newborns. Am. J. Obstet. Gynecol., 2011; 204 (Suppl.1): S13-S20

[25] Reece P.A.: Neuraminidase inhibitor resistance in influenza viruses. J. Med. Virol., 2007; 79: 1577-1586

[26] Schultsz C., Dong V.C., Chau N.V., Le N.T., Lim W., Thanh T.T., Dolecek C., de Jong M.D., Hien T.T., Farrar J.: Avian influenza H5N1 and healthcare workers. Emerg. Infect. Dis., 2005; 11: 1158-1159

[27] Siston A.M., Rasmussen S.A., Honein M.A., Fry A.M., Seib K., Callaghan W.M., Louie J., Doyle T.J., Crockett M., Lynfield R., Moore Z., Wiedeman C., Anand M., Tabony L., Nielsen C.F. et al.: Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA, 2010; 303: 1517-1525

[28] Takashita E., Meijer A., Lackenby A., Gubareva L., Rebelo-de-Andrade H., Besselaar T., Fry A., Gregory V., Leang S.K., Huang W., Lo J., Pereyaslov D., Siqueira M.M., Wang D., Mak G.C.: Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013-2014. Antiviral Res., 2015; 117: 27-38

[29] Thorlund K., Award T., Boivin G., Thabane L.: Systematic review of influenza resistance to the neuraminidase inhibitors. BMC Infect. Dis., 2011; 11: 134

[30] Treanor J.J., Hayden F.G., Vrooman P.S., Barbarash R., Bettis R., Riff D., Singh S., Kinnersley N., Ward P., Mills R.G.: Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. JAMA, 2000; 283: 1016-1024

[31] Varghese J.N., Smith P.W., Sollis S.L., Blick T.J., Sahasrabudhe A., McKimm-Breschkin J.L., Colman P.M.: Drug design against a shifting target: a structural basis for resistance to inhibitors in a variant of influenza virus neuraminidase. Structure, 1998; 6: 735-746

[32] Ward P., Small I., Smith J., Suter P., Dutkowski R.: Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. J. Antimicrob. Chemother., 2005; 55 (Suppl.1): i5-i25

[33] Whitley R.J., Hayden F.G., Reisinger K.S., Young N., Dutkowski R., Ipe D., Mills R.G., Ward P.: Oral oseltamivir treatment of influenza in children. Pediatr. Infect. Dis. J., 2001; 30: 127-133

[34] World Health Organization (WHO). Human infection with avian influenza A(H7N9) virus – China. http://www.who.int/csr/don/14-April-2015-avian-influenza-china/en/ (24 April 2015)

[35] World Health Organization (WHO). Avian influenza situation in Egypt – update, 18 January 2007. www.who.int/csr/don/2007 (24 April 2014)

[36] World Health Organization (WHO). Background and summary of human infection with influenza A(H7N9) virus. http://www.who.int/influenza/human_animal_interface/latest_update_h7n9/en/ (24 April 2014)

[37] World Health Organization (WHO). Issues new guidelines for the avian flu virus. www.who.int (24 April 2013)

[38] Yen H.L., Hoffmann E., Taylor G., Scholtissek C., Monto A.S., Webster R.G., Govorkova E.A.: Importance of neuraminidase active-site residues to the neuraminidase inhibitor resistance of influenza viruses. J. Virol., 2006; 80: 8787-8795

[39] Yu H., Feng Z., Uyeki T.M., Liao Q., Zhou L., Feng L., Ye M., Xiang N., Huai Y., Yuan Y., Jiang H., Zheng Y., Gargiullo P., Peng Z., Feng Y. et al.: Risk factors for severe illness with 2009 pandemic influenza A (H1N1) virus infection in China. Clin. Infect. Dis., 2011; 52: 457-465

[40] Yuen K.Y., Wong S.S.: Human infection by avian influenza A H5N1. Hong Kong Med. J., 2005; 11: 189-199