Understanding Influenza Virus Resistance to Antiviral Agents; Early Warning Signs for Wider Community Circulation

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(See the article by Hurt et al, on pages 148-57.)

Influenza viruses circulating among humans vary genetically from season to season and even within a season in different regions of the world. Thus, global surveillance for influenza viruses is critical to monitor for antigenic changes in circulating influenza viruses and to inform annual vaccine strain selection. In addition, surveillance is critical to monitor for virus susceptibility to antiviral drugs. Resistance to the adamantane class of drugs (amantadine and rimantadine), caused by substitutions at key amino acid residues in the M2 protein, has been widespread among influenza A viruses [1]. Adamantanes are not effective against currently circulating A(H3N2) viruses and 2009 pandemic influenza A(H1N1) (H1N1pdm09) viruses because of the S31N change in the M2 protein [2, 3].

Resistance to both approved neuraminidase (NA) inhibitors, oseltamivir and zanamivir, has been occasionally reported for all influenza virus A subtypes and influenza B viruses affecting humans [4]; however, widespread global circulation of influenza viruses resistant to an NA inhibitor (NAI) has occurred only once since the initiation of surveillance for NAI resistance in 1999 [5].

Early in the 2007-2008 influenza season, Norway reported the first cluster of seasonal influenza A(H1N1) viruses that carried the oseltamivir resistance–conferring H275Y substitution in the NA [6]. There was no association with prior oseltamivir exposure. Subsequently, during that season, many countries reported an increased prevalence of oseltamivir resistance among seasonal influenza A(H1N1) viruses, although prevalence varied globally [7-9]; previously, the prevalence had been <1%. During the next season (2008-2009), oseltamivir-resistant H275Y variant became the predominant seasonal influenza A(H1N1) viruses in the United States and other countries [10, 11].

During the 2009 pandemic, the oseltamivir-sensitive H1N1pdm09 virus replaced the oseltamivir-resistant seasonal influenza A(H1N1) virus. However, concerns of a repeat of the 2007-2009 phenomenon of rapid emergence and widespread circulation of oseltamivir-resistant viruses have accentuated the importance of ongoing global surveillance for NA inhibitor-resistant influenza viruses as well as the need to broaden therapeutic options.

Several investigators have suggested mechanisms that may have contributed to the replacement of the oseltamivir-susceptible seasonal influenza A(H1N1) virus with the oseltamivir-resistant viruses. First, substitutions in the NA, especially those in the active site or its proximity (such as H275Y), can decrease NA function, reducing viral fitness [4]; this was demonstrated for the H275Y substitution. Additional mutations in the NA of the 2008-2009 seasonal influenza A(H1N1) viruses (V234M, R222Q) were identified [12] that appeared to permit more normal NA function compared to H275Y viruses without these “permissive mutations” [13, 14]. Thus, permissive or complementary changes in the NA may have enabled the H275Y viruses to cocirculate with the oseltamivir-susceptible viruses in communities. However, they do not explain why the H275Y resistant viruses out-competed susceptible viruses, especially in the countries where oseltamivir was not widely used.

Second, other investigators reasoned that the H275Y substitution beneficially affected the hemagglutinin (HA) and NA functional balance in the seasonal influenza A(H1N1) viruses [15]; thus, the H275Y viruses might replicate better and transmit more efficiently, allowing the H275Y viruses to out-compete oseltamivir-susceptible viruses, even in the absence of drug pressure. Finally, the conventional mechanism for influenza viruses to continuously evolve and escape immune system defenses is antigenic drift in the major surface antigen HA. Although antigenic...
differences were not detected between the cocirculating oseltamivir-susceptible and resistant seasonal A(H1N1) viruses with the hemagglutination inhibition (HI) assay using ferret antisera. Investigators from Hong Kong have suggested that oseltamivir-resistant viruses may have been sufficiently different antigenically from oseltamivir-susceptible viruses when tested using human antisera [16]. Thus, oseltamivir-resistant viruses may have had the advantage of less population immunity compared with susceptible viruses. It is not possible to predict whether similar mechanisms will be important for H1N1pdm09 viruses, but these earlier studies provide a template to work from.

In this issue of the *Journal of Infectious Diseases*, Hurt et al. describe a cluster of 29 (15%) viruses with oseltamivir resistance among 191 H1N1pdm09-tested viruses from one region in Australia collected during May–September 2011. The prevalence of oseltamivir resistance among H1N1pdm09 viruses in other regions of Australia and the world was approximately 1% [17]. Among the patients infected with oseltamivir-resistant viruses, 97% had not received oseltamivir prior to specimen collection. The only feature common to all 29 patients in the cluster was residence within the Hunter New England (HNE) region. Epidemiology data suggest that the oseltamivir-resistant H1N1pdm09 viruses were circulating in the HNE community concurrently with oseltamivir-susceptible viruses, unrelated to drug pressure. We do not know whether clinical illnesses caused by the oseltamivir-resistant H1N1pdm09 viruses were similar to that of oseltamivir-susceptible viruses; data from a sample of patients infected with oseltamivir-susceptible viruses could have provided further insight into virus fitness but, unfortunately, were not collected.

The viruses characterized in this article were highly similar genetically and contained two other NA substitutions, V62I and N386S, which were absent from most of the oseltamivir-susceptible H1N1pdm09 viruses in Australia. Both susceptible and resistant viruses had V241I and N369K substitutions. A computational analysis of NA protein stability suggested that V241I and N369K would improve protein stability in a NA with H275Y by 50%, and that addition of N386S would further stabilize the protein and possibly improve oseltamivir-resistant virus fitness. Substitutions N386S/Y/K, leading to a loss of a potential glycosylation site, have been seen in viruses circulating in many countries during the 2011–2012 season. The cluster of oseltamivir-resistant H1N1pdm09 viruses in Australia did not spread beyond the HNE region. Was this because the influenza season was ending and the cluster virus did not have a chance to be transmitted more widely? Will we see an increasing prevalence in H275Y H1N1pdm09 viruses globally during a future season? Whether the necessary factors for an evolutionary advantage over the susceptible virus have been met by these new H275Y H1N1pdm09 viruses is unclear at this time. As Hurt et al. stress, it is essential to continue close monitoring of H1N1pdm09 viruses for NA substitutions associated with oseltamivir resistance, such as H275Y and other NA substitutions, that may play an important role in offsetting any deleterious effect of the H275Y mutation on virus fitness, as well as changes in other virus proteins that might suggest a divergent evolution of resistant and susceptible viruses.

Widespread community circulation of a virus resistant to both adamantanes and to oseltamivir has many implications, most notable for clinical care. Inhaled zanamivir would be the only approved treatment option in many countries, an option not available to children aged <7 years, persons with underlying lung conditions, patients on mechanical ventilation, and patients with severe influenza infection [18]. Currently, intravenous zanamivir, an experimental drug undergoing phase III clinical trials, would be the most appropriate treatment choice for patients who were severely ill from an infection with an adamantane- and oseltamivir-resistant virus. However, until intravenous zanamivir is approved, availability is limited to experimental use and clinical trials. In the United States, emergency use authorizations are only possible during public health emergencies and fail to provide data that are useful toward Food and Drug Administration approval of a new drug. Thus, improving availability of an experimental drug for severely ill patients in the event of widespread circulation of an adamantane- and oseltamivir-resistant virus would be difficult.

Given the paucity of treatment options and hurdles that may be involved to use an experimental drug more widely, it is imperative to learn more about drug resistance in H1N1pdm09 viruses. Susceptibility to NA inhibitors is determined by the in vitro neuraminidase inhibition (NI) assay, which determines the median inhibitory concentration (IC\textsubscript{50}), the concentration of drug required to inhibit NA activity by 50%. NI assay IC\textsubscript{50} values vary for each virus type and subtype. In addition, the IC\textsubscript{50} value associated with oseltamivir resistance can vary for different influenza A subtypes [4]. A few studies have tried to correlate laboratory-measured NA resistance with clinical outcomes in patients infected with seasonal influenza A (H1N1) viruses with the H275Y substitution [19–22]. As Hurt et al. discuss, the IC\textsubscript{50} values for oseltamivir-resistant H1N1pdm09 viruses are lower than for oseltamivir-resistant seasonal A(H1N1) viruses and may be within the range achievable in plasma with oral dosing. This raises the question of whether oseltamivir might retain some clinical effectiveness against infections caused by H1N1pdm09 virus with the H275Y substitution and that oseltamivir might remain a treatment option, at least for persons without severe illness.

The prevalence of oseltamivir-resistant H1N1pdm09 viruses was low during 2009–2010, and appeared to occur most frequently during treatment and in
patients with underlying immunosuppressive conditions [23, 24]. Surveillance from 2010–2011 suggested that community circulation of oseltamivir-resistant H1N1pdm09 viruses unrelated to oseltamivir use may be increasing, although prevalence was still approximately 1% [25, 26]. The report by Hurt et al. raises concern about more widespread community circulation of an adamantane- and oseltamivir-resistant H1N1pdm09 virus. But is it inevitable? Efforts to learn as much as possible about these new viruses, including permissive mutations and antigenic drift, as well as Public Health Threats. Situations where oseltamivir-resistant and susceptible viruses cocirculate in the same season may offer unique opportunities to compare both viruses and patients infected by both viruses. Surveillance, and the prompt identification and investigation of clusters, may provide both an early warning for future trends of virus changes and the opportunity to learn about viruses with significant potential to cause public health threat.

Notes

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References