Emerging Influenza Antiviral Resistance Threats

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(See the articles by Sheu et al, on pages 13–17, Moore et al, on pages 18–24, and Pizzorno et al, on pages 25–31.)

Antiviral resistance in influenza may not only develop during treatment but also sometimes transmit widely to replace susceptible strains in the absence of drug pressure. This transmission is exemplified by the global spread of adamantane-resistant A(H3N2) viruses since 2003, oseltamivir-resistant seasonal A(H1N1) viruses since 2007, and adamantane-resistant pandemic A(H1N1) viruses in 2009, events that emphasize the unpredictability of influenza viruses and the increasing challenges of clinical management of influenza, especially given the current paucity of therapeutic choices. Three articles in this issue of *The Journal of Infectious Diseases* extend these observations, describing the plasticity of seasonal A(H1N1) viruses in developing dual adamantane-oseltamivir resistance [1], the effects on drug susceptibility and viral fitness of various neuraminidase (NA) mutations in pandemic A(H1N1) viruses [2], and the risk of nosocomial spread of oseltamivir-resistant pandemic A(H1N1) virus [3]. Together, these findings illustrate that single reassortment events or mutations can lead to the emergence of transmissible variants of pandemic 2009 or seasonal A(H1N1) viruses unresponsive to most, if not all, of our currently available drugs. This raises multiple questions regarding patient management and preparations for future influenza outbreaks.

High-level adamantane resistance rapidly develops during treatment and is conferred by single amino acid changes in the M2 ion channel, most notably at S31N, that do not diminish viral replication or transmissibility [4, 5]. Until several years ago, wide-scale spread of oseltamivir-resistant viruses associated with NA mutations, such as the H275Y change that causes marked loss of oseltamivir susceptibility in N1-containing viruses [6], was deemed unlikely in view of observed reductions in viral infectivity and virulence associated with the H275Y change in earlier seasonal H1N1 viruses [7, 8]. However, this was clearly not the case for the oseltamivir-resistant A/Brisbane/59/07(H1N1) virus, which transmitted very efficiently from person to person, ultimately replaced susceptible viruses in the absence of selective drug pressure in much of the world, and caused illness similar to that caused by susceptible viruses, including severe disease in at-risk patients [9–11]. The mechanisms for the undiminished fitness of oseltamivir-resistant A/Brisbane/59/07(H1N1) virus are incompletely understood, but the reduced substrate affinity of the NA with H275Y may have restored a balance between hemagglutinin (HA) receptor-binding and NA receptor-cleaving functionalities altered by earlier NA mutations that increased substrate affinity [12–14]. Further evidence of this hypothesis was provided by recent studies showing that NA mutations at sites outside of the active enzyme site enhanced surface expression of properly folded NA and appeared to facilitate the subsequent emergence of the H275Y mutation, possibly by restoring the functional balance between HA and NA [15]. Worryingly, these observations suggest the emergence of drug-resistant influenza virus variants with higher transmission fitness than that of the corresponding susceptible virus by selective pressures apparently unrelated to antiviral exposure.

Of concern in this respect is the finding by Pizzorno and colleagues [2] that an I223V NA change not only increased oseltamivir and peramivir resistance of pandemic A(H1N1) virus conferred by the H275Y NA change,

Received and accepted 23 September 2010.

FGH: No research grants or personal honoraria from industry for lectures, consulting, or other activities since 2006.

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(See the articles by Sheu et al, on pages 13–17, Moore et al, on pages 18–24, and Pizzomo et al, on pages 25–31.)

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The Journal of Infectious Diseases 2011;203:6–10

1537-6613/2011/2031-0001$15.00
DOI: 10.1093/infdis/jiq012

similar to previous findings for seasonal A(H1N1) and avian A(H5N1) viruses [16], but also restored NA substrate affinity and replication fitness in vitro. Of note, Garrison and coauthors identified pandemic A(H1N1) virus infections with dual H275Y and I223V NA mutations in a pair of campers [17], and it is tempting to speculate whether the I223V change fostered transmission in this event. An I223R mutation in pandemic N1 is associated with 7- to 10-fold decreased susceptibility to zanamivir and up to 45-fold decreased susceptibility to oseltamivir [18–20]; the same mutation in conjunction with H275Y causes further reductions in NA inhibitor (NAI) susceptibility, especially for oseltamivir and peramivir [19, 21, 22]. Variants harboring the I223R change emerged and replicated to high titers during intravenous zanamivir treatment in 1 severely immunocompromised patient in whom H275Y had developed during a previous course of oseltamivir treatment [18]. In another immunocompromised host, the I223R mutation emerged during oseltamivir therapy but became predominant after subsequent inhaled administration of zanamivir [19]. Further studies of the fitness effects of I223 mutations, alone and in conjunction with H275Y in animal models, and close monitoring of changes in the I223 residue in N1 viruses are clearly needed. In addition, because other mutations may confer reduced susceptibility to 1 or more NAIs in N1-containing [21–26] and other influenza viruses, continued surveillance using phenotypic assays in addition to sequence-based ones remains essential.

Complicating things further, dual adamantane-oseltamivir resistance may emerge and spread by reassortment or mutation. The recent cocirculation of different genetic groups of seasonal A(H1N1) viruses with different antiviral-susceptibility patterns, notably oseltamivir-resistant clade 2B A/Brisbane/59/07(H1N1)-like and adamantane-resistant clade 2C viruses, has fostered the emergence of dually resistant variants. Sporadic dually resistant clade 2B and 2C variants were reported in Cambodia in 2007 and Hong Kong in 2008 [27, 28]. Dually resistant clade 2B A/Brisbane/59/07-like viruses that acquired an M gene harboring the S31N mutation from clade 2C were again detected in Hong Kong in April 2009 and became predominant within 2 months [29]. Sheu and colleagues confirm that several routes to dual resistance have occurred in seasonal A(H1N1) viruses, including by exchange of M and NA genes between clade 2B and 2C variants, by emergence of adamantane-resistance mutations in oseltamivir-resistant viruses with drug treatment, by transmission from others, or perhaps spontaneously [1]. To date, oseltamivir resistance in pandemic A(H1N1) viruses due to acquisition of the NA from seasonal A(H1N1) virus has not emerged, although such variants have been generated in the laboratory [30, 31], and co-infections by pandemic A(H1N1) and oseltamivir-resistant seasonal A(H1N1) viruses have been documented [32]. Also human infections by oseltamivir-resistant reassortant A(H1N1) viruses harboring the HA and NA genes from an A/Brisbane/59/07(H1N1)-like virus and the internal genes from a triple-reassortant North American swine [33] and reassortants of swine influenza virus and pandemic A(H1N1) viruses [34] have been reported, raising the possibility that swine may severe as source for novel viruses with antiviral resistance. One recent study found reduced replication and transmission of a reassortant pandemic A(H1N1) virus containing the oseltamivir-resistant NA from a seasonal A(H1N1) virus in ferrets [35], but another found enhanced infectivity in mice [31], so more work is needed. Fortunately, seasonal A(H1N1) viruses have been largely replaced by the pandemic A(H1N1) virus, which diminishes the likelihood that such oseltamivir-resistant reassortants will emerge in the future. However, the potential for resistant variants emerging in nonhuman hosts and for rapid changes in local antiviral-susceptibility patterns emphasizes the need for timely monitoring at both community and global levels.

Because of pre-existing adamantane resistance (S31N mutation in M2) in almost all pandemic A(H1N1) viruses, acquisition of oseltamivir resistance inevitably leads to dual resistance in this virus. To date, oseltamivir resistance in pandemic A(H1N1) viruses has been related almost exclusively to acquisition of the H275Y mutation during treatment, especially in immunocompromised hosts, or less often during prophylaxis, and has been uncommon (304 cases confirmed worldwide as of August 2010) [36, 37]. Even in Japan, where the largest per capita amounts of oseltamivir were used during the pandemic response, approximately 1% of more than 6000 isolates possessed the H275Y mutation, although 9 of 69 were from patients not exposed to oseltamivir to suggest possible community transmission [20]. In studies employing sequential virologic sampling, the risk of H275Y emerging in oseltamivir-treated outpatients has been low (0 to $<2\%$) [38–42], much less than that reported previously in studies of children infected with seasonal A(H1N1) viruses [43]. Except in immunocompromised hosts, resistance rates also appear relatively low in hospitalized patients [44–46]. Whereas these observations may be somewhat reassuring, infections with oseltamivir-resistant pandemic A(H1N1) viruses have caused typical influenza illnesses, including severe and fatal disease, particularly in immunocompromised hosts [36, 47, 48]. Not surprisingly, immunocompromised hosts and, less often, apparently, immunocompetent patients hospitalized with pneumonia have the highest risks of developing oseltamivir resistance during treatment [44, 46–49]. Moreover, in lymphopenic patients, resistant virus may persist for weeks after cessation of oseltamivir treatment, providing a reservoir for possible transmission to other

high-risk patients and indicating that such patients should be a high priority for targeted susceptibility monitoring [3, 44, 47, 50]. Indeed, oseltamivir-resistant pandemic A(H1N1) variants may be transmitted efficiently, as exemplified by reports of nosocomial transmission both by Moore and colleagues [3] and in another hematology-oncology unit [36], by another reported cluster of infection in the community [51], and by the finding that a substantial fraction (about 8–10%) of resistant variants have been recovered from untreated persons with no epidemiological link [37, 52]. As in an earlier report of nosocomial transmission of oseltamivir-resistant seasonal A(H1N1) [10], the routes of transmission in the report by Moore and colleagues were not clear, and healthcare workers might have played a role. Such observations highlight the importance of stringent infection-control practices and mandatory influenza immunization of healthcare workers involved in the care of high-risk patients.

The threat of resistance poses major challenges in clinical management of seasonal and pandemic influenza. Monitoring of viral clearance during treatment of influenza in immunocompromised and seriously ill hospitalized patients is needed. Unfortunately, options for rapid detection of resistance are currently limited, and most available assays target only the H275Y mutation. When resistance emergence is not recognized, prolonged use of suboptimal or ineffective therapy may lead to selection of highly resistant viral populations and potentially other mutations. Consequently, decisions often need to be based on clinical and virologic responses, and switches to other antivirals made empirically. The choice of alternative antivirals in these situations is very limited. The high prevalence of adamantane resistance in circulating influenza A viruses indicates no role for these agents at present, outside of clinical trials. Zanamivir is currently the only widely approved agent with antiviral activity against oseltamivir-resistant A(H1N1) viruses. The commercial form is likely adequate for prophylaxis, especially in management of outbreaks involving immunocompromised hosts and treatment of uncomplicated illness [53], but the safety and effectiveness of orally inhaled or nebulized zanamivir have not been established in more seriously ill patients, and the lactose carrier can interfere with ventilator filters [54]. Intravenous NAIs provide reliable drug delivery in such patients but are currently investigational in most countries. Unlike zanamivir, peramivir is 57- to 263-fold less inhibitory for pandemic A(H1N1) viruses with the H275Y mutation in enzyme inhibition assays [2, 47]. Whereas multiple peramivir doses were inhibitory for 1 laboratory A(H1N1) strain with this mutation in mice [55], single doses appeared to be no better than a 5-day oseltamivir regimen in oseltamivir-resistant seasonal A(H1N1)-infected adults [56] and failed to reduce replication in 1 immunocompromised patient with oseltamivir-resistant pandemic A(H1N1) illness [47]. More data are needed before peramivir may be considered for use in high-risk persons with suspected or proven oseltamivir-resistant A(H1N1) infections, and intravenous zanamivir presently would be the most reasonable antiviral choice when oseltamivir-resistance is proven or suspected in seriously ill pandemic A(H1N1) patients [3, 57–60].

Combination antiviral therapy makes strong theoretical sense in efforts to rapidly control replication and reduce the emergence of resistance, particularly in immunocompromised and seriously ill patients. However, the current therapeutic armamentarium approved for use in combinations is limited, and there are no published data from adequately powered, controlled clinical trials. In murine models of adamantane-resistant A(H5N1) infection, the addition of amantadine to oseltamivir was no more effective than oseltamivir monotherapy [61, 62]. Combinations of oseltamivir and zanamivir have been used in individual patients [3], but concentration-dependent additivity to antagonism has been reported in vitro for pandemic A(H1N1) strains [63], and a recent randomized study in A(H3N2)-infected ambulatory patients indicated that the antiviral and clinical efficacies of the combination of oral oseltamivir and inhaled zanamivir were similar to inhaled zanamivir alone and inferior to oral oseltamivir alone [64]. Consequently, more pre-clinical studies are needed to determine the antiviral activities of this combination for both oseltamivir-susceptible and oseltamivir-resistant strains. Oseltamivir combined with systemic ribavirin shows primarily additive antiviral effects in murine models [62, 65]. Interestingly, a triple-drug regimen of oseltamivir, ribavirin, and amantadine was reported to show synergy in vitro for adamantane- or oseltamivir-resistant strains [63], and unpublished data indicate survival benefits in mice infected with an amantadine-resistant pandemic A(H1N1) strain, compared to oseltamivir monotherapy and dual combinations. Although the mechanisms remain unclear, initial clinical studies of this regimen are in progress. Of note, illness in 1 highly immunocompromised patient progressed despite the use of the triple-drug regimen [66]. Promising investigational agents to be studied in combination with NAIs include the polymerase inhibitor favipiravir, the receptor-destroying sialidase DAS181, broadly reactive heterosubtypic monoclonal antibodies, and convalescent plasma or hyperimmune globulin. However, there is also a need for developing new influenza antivirals with novel mechanisms of action. The design of future clinical trials needs to be guided by a better understanding of the relationships between viral-replication measures at different sites in the respiratory tract, disease pathogenesis biomarkers such as plasma cytokines and chemokines, and clinical outcomes [67]. Such information will ensure more rapid development and testing of alternative

antiviral regimens for use in immunocompromised hosts and seriously ill hospitalized patients to address their unmet medical needs and the associated public health concerns, particularly the continuing threat of antiviral resistance.

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