Detection of an influenza B strain with reduced susceptibility to neuraminidase inhibitor drugs.

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Neuraminidase inhibitors (NAIs), oseltamivir and zanamivir have played an essential role in the prophylaxis and treatment of influenza. The residues forming the NA active sites are conserved among A and B influenza viruses (3). Conserved residues are in direct contact with the substrate or provide structural framework for the functional residues. There have been reports of in vivo resistance for B viruses (4,7). Here we report the isolation of a novel influenza B virus with reduced sensitivity to NAIs.

On December 22, 2010, an 87 year old woman presented to an Ontario hospital with an influenza-like illness. Symptoms began on December 19, 2010. She was admitted to hospital and treated with oseltamivir for 5 days (75 mg twice daily) making an uneventful recovery. Influenza B was detected in a nasopharyngeal swab collected on December 22, 2010. The specimen was cultured in rhesus monkey kidney cells and the isolate was designated B/Ontario/RV75-11/2010. Susceptibility of B/Ontario/RV75-11/2010 to NAIs was determined by a chemiluminescent neuraminidase inhibition assay. The IC$_{50}$ values for B/Ontario/RV75-11/2010 showed a 7- to 13-fold and a 6- to 18-fold increase compared to the wild type control B/Hong Kong/36/2005 for oseltamivir and zanamivir, respectively (Table 1). Specimen collection and drug treatment initiation occurred on the same day, indicating that the reduced sensitivity may have occurred naturally.

Sequencing of the NA gene showed a G109E and a N340D substitution compared to the reference strain B/Brisbane/60/2008. The N340D substitution has been found in NAI-susceptible strains of influenza B circulating in Canada. In contrast, the G109E substitution is unique to B/Ontario/RV75-11/2010. To determine if the G109E mutation was responsible for the reduced susceptibility to NAIs, we tested another Canadian isolates with identical NA sequence as B/Ontario/RV75-11/2010, except for the G109E
mutation. B/Ontario/RV535/2011 was susceptible to oseltamivir and zanamivir (Table 1). To our knowledge, this is the first report linking a change at residue 109 to reduced susceptibility to NAIs. The mechanism by which this change leads to reduced susceptibility to NAIs is unknown. Residue 109 is not one of the highly conserved residues that form the NA active site. However, it is located near residue R118 that interacts with sialic acid and E119 that provides structural framework for the active site (1). It has been reported that substitutions in NA at positions that confer resistance to NAIs may compromise enzyme function and result in reduced enzyme stability (5,6), instability of the NA tetramer (2), or a change in the optimum pH for NA activity (5). Further research is needed to determine the mechanism by which a G109E substitution alters susceptibility to NAIs.

Since the patient recovered without complication, the clinical significance of the G109E substitution may be limited, but remains to be determined. The recovery of the influenza B virus with the new G109E substitution which affects susceptibility to two drugs available for treatment of influenza B virus infections highlights the importance of monitoring NAI- susceptibility using functional assays.


Table 1: Drug susceptibility and genotype of influenza B/Ontario/RV75-11/2010 virus.

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Passage of isolate</th>
<th>NA change</th>
<th>IC₅₀ (nM)*</th>
<th>Oseltamivir Fold</th>
<th>Zanamivir Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ontario/RV75-11/2010</td>
<td>1</td>
<td>G109E</td>
<td>19.87 ± 0.41</td>
<td>7</td>
<td>23.11 ±1.57</td>
</tr>
<tr>
<td>B/Ontario/RV535/2011</td>
<td>1</td>
<td>G109</td>
<td>3.60</td>
<td>1.2</td>
<td>6.37</td>
</tr>
<tr>
<td>B/Hong Kong /45/2005</td>
<td>Susceptible control</td>
<td>WT</td>
<td>3.00±0.47</td>
<td>1</td>
<td>3.83±0.34</td>
</tr>
<tr>
<td>B/Hong Kong /36/2005</td>
<td>Resistant control</td>
<td>R371K</td>
<td>633.33±185.59</td>
<td>211.93±89.38</td>
<td></td>
</tr>
</tbody>
</table>

*Susceptibility to oseltamivir and zanamivir was determined by a chemiluminescent neuraminidase inhibition assay, using the NA-Star® kit (Applied Biosystems Inc.). NA inhibition was assayed with viruses standardized to equivalent NA enzyme activity and incubated with NAI at concentrations of 0.0316 nM to 1000 nM. The 50% inhibitory concentration (IC₅₀) was calculated by plotting the percentage inhibition of NA activity against the inhibitor concentration, using GraphPad PRISM 4 software for curve fitting.