Antiviral Activity of Enisamium Against Influenza Viruses in Differentiated Normal Human Bronchial Epithelial Cells

David Boltz, Xinjian Peng, Miguel Muzzio, Pradyot Dash, Paul Thomas, Rajendra Mehta, and Victor Margitich

1) IIT Research Institute, Chicago, IL, USA; 2) St. Jude Children Research Hospital, Memphis, TN, USA; 3) Farmak Joint Stock Company, Kiev, Ukraine

Influenza is an acute respiratory illness caused by influenza A and B viruses that occurs as annual epidemics with significant morbidity and mortality and occasional pandemics. Although vaccines have proven effective in mitigating the impact of influenza epidemics, due to possible antigenic mismatch and significant time required to develop a clinically available vaccine against a new virus strain, effective antiviral therapies are essential for immediate intervention against influenza infections. Currently, only a single class of antiviral drugs (e.g. neuraminidase [NA] inhibitors oseltamivir [Tamiflu®, Roche] and zanamivir [Resepron®, GlaxoSmithKline]) is recommended for prophylaxis and treatment of influenza in adults and children worldwide.

Efforts are focused on identifying new treatment options and drug targets to eliminate the pathogenic properties of influenza virus. To this end, some drugs currently marketed in the countries of the former Soviet Union were reported to exert antiviral activity against influenza A and B viruses. One of these drugs is enisamium iodide, or Amizon, a licensed and marketed in Russia, Ukraine, Belarus, Kazakhstan and Uzbekistan as an antiviral agent against influenza (Margitich, personal communication).

Figure 1. Chemical structure of enisamium [[4-Benzylcarbamoyl]1-methylpyridin-1-ium] iodide

### MATERIALS & METHODS

**Cell Culture:** MatTek’s EpAirway System (MatTek, Ashland, MA) differentiated normal human-derived bronchial epithelial cells (NHBE) were used for the study. The cells from a single donor were used for assay consistency. The apical surface of the cells was exposed to a humidified 95% air/5% CO2 environment and the basolateral medium changed and mucin washed every 24-48 hours.

**Test Materials:** The test article, enisamium, was provided by the Farmak JSC. The positive control oseltamivir carboxylate was obtained from Toronto Research Chemicals, TRC (Toronto, Canada).

**Virus Challenge:** NHBE cells were inoculated with influenza A viruses by exposure of the apical side to influenza virus. After a 1-hour incubation, the viral inoculum was removed from the cells, the apical side of the cells washed with Phosphate Buffered Saline (PBS). Test Material Administration: For the positive control, oseltamivir carboxylate, NHBE cultures were exposed on the basal side to oseltamivir for 60 minutes, prior to viral inoculation. Enisamium or control media was added to the basal side of the NHBE culture system prior to or post inoculation and incubated for the indicated duration of the experiment.

### RESULTS

**Antiviral Activity of Enisamium Chloride Against Influenza A and B Viruses in Differentiated NHBE Cells**

![Influenza virus titers (log2 TCID50/mL ± SD)](image)

- **Drug, dose (µM)**
  - A/Quebec/20/06 (H1N1) 2007: 0, 6.0 ± 0.4, 3.75 ± 0.3 (A), 3.0 ± 0.4 (B), 4.9 ± 0.3 (C)
  - A/Brisbane/59/07 (H3N2): 0, 6.5 ± 0.0 (A), 6.2 ± 0.3 (B), 6.2 ± 0.6 (C)
  - A/T/0/99 (HIN1): 0, 6.0 ± 0.4 (A), 6.2 ± 0.6 (B), 6.2 ± 0.6 (C)
  - A/Perth/16/09 (H3N2): 0, 6.0 ± 0.3 (A), 6.2 ± 0.6 (B), 6.2 ± 0.6 (C)
  - B/Texas/06/11: 0, 6.5 ± 0.0 (A), 6.2 ± 0.3 (B), 6.2 ± 0.6 (C)

<table>
<thead>
<tr>
<th>Drug, dose (µM)</th>
<th>A/Quebec/20/06 (H1N1) 2007</th>
<th>A/Brisbane/59/07 (H3N2)</th>
<th>A/T/0/99 (HIN1)</th>
<th>A/Perth/16/09 (H3N2)</th>
<th>B/Texas/06/11</th>
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<tr>
<td>0</td>
<td>6.0 ± 0.4 (A)</td>
<td>6.5 ± 0.0 (A)</td>
<td>6.0 ± 0.4</td>
<td>6.5 ± 0.0</td>
<td>6.5 ± 0.0</td>
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<tr>
<td>6.0 ± 0.4 (B)</td>
<td>3.75 ± 0.3 (A)</td>
<td>6.2 ± 0.6</td>
<td>4.9 ± 0.3</td>
<td>6.2 ± 0.6</td>
<td>6.2 ± 0.6</td>
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<tr>
<td>6.0 ± 0.4 (C)</td>
<td>3.0 ± 0.4 (A)</td>
<td>3.6 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>4.9 ± 0.3</td>
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**Suspension of oseltamivir carboxylate was 1 µM.**

**Concentration of oseltamivir carboxylate was 1000 µM in differentiated NHBE cells at 37°C under 5% CO2 for 24 hours in serum-free Bronchial Epithelial Cell Growth Medium.**

**Effect of Pre-incubating NHBE Cells with Enisamium Prior to Viral Inoculation**

![Effect of Pre-incubating NHBE Cells with Enisamium Prior to Viral Inoculation](image)

**Inhibitory Activity of Enisamium Against Different MOIs of A/Brisbane/59/07 (H1N1) Influenza Virus**

- **(A) MOI of 0.01 PFU/cell**
- **(B) MOI of 0.001 PFU/cell**
- **(C) MOI of 0.0001 PFU/cell**

**Permeability of Enisamium in Differentiated NHBE Cells**

<table>
<thead>
<tr>
<th>Drug, dose (µM)</th>
<th>Concentration in NHBE cells (mean ± SD)</th>
<th>Permeability (%)</th>
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<tr>
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<tr>
<td>10</td>
<td>30.8 ± 3.8</td>
<td>1.4</td>
</tr>
<tr>
<td>50</td>
<td>213 ± 34</td>
<td>1.6</td>
</tr>
<tr>
<td>100</td>
<td>410 ± 77</td>
<td>1.6</td>
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<tr>
<td>500</td>
<td>1727 ± 108</td>
<td>1.3</td>
</tr>
<tr>
<td>1000</td>
<td>3009 ± 132</td>
<td>1.1</td>
</tr>
</tbody>
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**Effect of Time-of-Enisamium Addition on Inhibition of Influenza Virus Replication in NHBE Cells**

![Effect of Time-of-Enisamium Addition on Inhibition of Influenza Virus Replication in NHBE Cells](image)

In conclusion, we have reported antiviral activity of an anti-influenza compound, enisamium, supporting the reported clinical efficacy. Although the mechanism of action of enisamium has yet to be identified, data presented here indicates that enisamium targets viral replication of influenza viruses. Enisamium was added before or shortly after infection, the reduction in M gene expression and viral titers by enisamium indicates that enisamium inhibits viral RNA synthesis. Interestingly, viral titers continued to increase in the presence of enisamium; therefore, enisamium suppressed influenza virus replication but did not completely inhibit it. Further exploration is required to provide a better understanding of the mechanism of action.

**ACKNOWLEDGEMENTS**

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