Influenza A Virus: Emergence of Drug Resistance & Quest for Solutions

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Influenza A Virus: A Global Threat

http://cagle.msnbc.com/news/BirdFlu05/1.asp
## Statistics - WHO

<table>
<thead>
<tr>
<th>Flu Type</th>
<th>Infected</th>
<th>Human Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1918 Spanish</td>
<td>-</td>
<td>&gt; 50 Million</td>
</tr>
<tr>
<td>1957 Asian</td>
<td>-</td>
<td>&gt; 2 Million</td>
</tr>
<tr>
<td>1968 Hong Kong</td>
<td>-</td>
<td>&gt; 1 Million</td>
</tr>
<tr>
<td>2003 Avian (H5N1)</td>
<td>-</td>
<td>262</td>
</tr>
<tr>
<td>2009 Swine (H1N1)</td>
<td>1.5 Million</td>
<td>&gt;24,000</td>
</tr>
<tr>
<td>Next?</td>
<td></td>
<td></td>
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</tbody>
</table>

http://www.flucount.org/
Influenza Virus

- A negative-sense single-stranded RNA virus of family Orthomyxoviridae
- Three different genera: A, B and C

Influenza Virus

Virus envelope proteins

- Hemagglutinin
  - 16 subtypes
    - H1-H16

- Neuraminidase
  - 9 subtypes
    - N1-N9

Group 1
- N1, N4, N5 & N8
- H1N1, H5N1

Group 2
- N2, N3, N6, N7 & N9
- H3N2
Influenza Life Cycle

von Itzstein M., Nature Reviews Drug Discovery. 2007, 6, 967-974
Strategies to Combat Influenza Virus

- Vaccination
  - Neutralized viral antigens
  - Induces immunity against a virus

- Chemotherapy
  - Chemical drugs
  - Inhibit the viral life cycle

Bhattacharya P. et al., *J. Virology* 2010, 84, 361-371
Effective Vaccination?

- **Vaccine effectiveness**
  - Correct prediction of circulating viruses
  - Limited by time constraint of manufacturing process

- **H1N1 2009**
  - Different from ordinary Influenza A viruses
  - Genetic material; from different viral strains
  - Radically different phenotypes

Bhattacharya P. et al., *J. Virology* 2010, 84, 361-371
Commercially Available Common Chemotherapeutics

Neuraminidase Inhibitors

- Zanamivir (Relenza®)
- Oseltamivir (Tamiflu®)

M2 Ion Channel Blockers

- Amantadine (Symetrel®)
- Remantadine (Flumadine®)

Emergence of Drug Resistance

- M2 ion channel blockers
  - Amantadine and Rimantadine

- In 2003-06 pandemic outbreak, avian influenza subtypes show resistance to Amantadine

- Amantadine has not been recommended as a pandemic control drug since 2005 in USA

Emergence of Drug Resistance

- Neuraminidase Inhibitors (NAI)
  - Zanamivir (Relenza®), Oseltamivir (Tamiflu®)
    – Discovered in 1995 & 1997

- Resistance in 16-18% of influenza patients

Resistance!!! How?

- **Antigenic Drift**
  - Point mutations viral genome
  - Resistant mutant strains

- **Antigenic Shift**
  - Reassortment of RNAs between different species (eg: H1N1 2009)
  - Different antigenic determinants
How Should We Face the Problem?

- Stop antigenic drift or antigenic shift? No
- Modify existing drugs? May be
- Discover novel drugs? Challenging
- Look for novel drug targets? Challenging
Drug Targets in Influenza Life Cycle

- **M2 ion channel Blockers**
  - Eg: Amantadine, Rimantadine

- **Neuraminidase Inhibitors**
  - Eg: Tamiflu®, Relenza®

- **Hemagglutinin Inhibitors**
  - Eg: BMY27709

- **RNA polymerase Inhibitors**
  - Eg: Ribavirin

*von Itzstein M. et al., Nature Reviews Drug Discovery. 2007, 6, 967-974*
Outline

- M2 ion channel
  - M2 Ion Channel Inhibition
  - Emergence of Resistance
  - Novel Ion Channel Inhibitors

- Neuraminidase Enzyme
  - Enzyme Mechanism
  - Enzyme Inhibition
  - Emergence of Resistance
  - Novel Neuraminidase Inhibitors
Mechanism of M2 Ion Channel

- Homotetrameric transmembrane domain protein
- His37 – Proton sensor
- Trp41 – Gate

How Do Adamantane Based Drugs Inhibit M2 Ion Channel?

- Two proposed mechanisms
  - Allosteric inhibition
  - Channel pore blocking inhibition
Allosteric Inhibition

NH₂

NH₂

Amantadine
Rimantadine

Transmembrane channel

C-terminal base

~30 Å

What Happens in Resistant Mutants?

- S31N, in 99.9% of resistant viruses in 2005/06 flu season
  - Wild type- IC$_{50}$ = 16 µM
  - S31N- IC$_{50}$ = 199.9 µM

- Other mutations; L26F, V27A, A30T, G34E and L38F

Val27, Ala30 and Ser31 make a pocket to accommodate Amantadine

What Happens in Resistant Mutants?

- S31N mutation gives rise to structural changes in the M2 transmembrane domain (M2TMD):
  - Extensive H-bonding between Asn carboxamides
  - High polarity

M2TMD Structure Reveals Clues to Evolve Novel Drugs

- Aqueous pore created, near highly conserved His37-Trp41 region

- Drugs, binding to the N-terminal of the channel pore will not be successful due to frequent mutations

- Drugs, which can bind to the altered allosteric site of mutants will be successful

Spiro[5,5]undecane Compounds

- Spiranamine - potential drug against L26F & V27A mutants
- L26 - critical packing residue at the helix-helix interface
- V27 - projects directly towards the pore

<table>
<thead>
<tr>
<th>Ion Channel</th>
<th>Spiranamine IC$_{50}$ (µM)</th>
<th>Amantadine IC$_{50}$ (µM)</th>
<th>Spiro-piperidine IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/M2</td>
<td>12.6</td>
<td>15.8</td>
<td>0.92</td>
</tr>
<tr>
<td>A/M2 L26F</td>
<td>30.6</td>
<td>164.4</td>
<td>-</td>
</tr>
<tr>
<td>A/M2 V27A</td>
<td>84.9</td>
<td>1840.0</td>
<td>-</td>
</tr>
<tr>
<td>A/M2 S31N</td>
<td>&gt;10 mM</td>
<td>237.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Neuraminidase (NA) Enzyme

- Homotetrameric glycoprotein anchored in viral membrane
- Hydrolysis of glycosidic linkage between sialic acid and glycoprotein

Neuraminidase Mechanism

Neuraminidase Inhibitors

- **Relenza®**
  - Inhaled / intranasal treatment
  - Low bioavailability

- **Tamiflu®**
  - Oral drug
  - High bioavailability

Binding of Relenza® to the NA Active Site

- **Relenza®**
  - Glu119 – Guanidium gp
  - $K_i = 5 \times 10^{-8} \text{M}$

Binding of Tamiflu® to the NA Active Site

- Tamiflu®
- Glu276 - Arg224 ionic interactions
- $K_i = 2 \times 10^{-10} \text{ M}$

Sialic Acid

Mutations in NA Active Site

- H274Y in NA, 99.4% of all isolated Tamiflu® resistant H1N1 viruses in USA in 2009

- Tamiflu® activity loss by 900-2500 fold

- Q136K in NA; Relenza® resistant Influenza strain – H1N1

- Relenza® activity loss by 300 fold

Hurt A.C., Holien J.K, Barr G.I., AAC 2009, 53, 4433-4440
Nguyen J.T. et al., AAC 2009, 53, 4115-4126
Mechanisms of Drug Resistance

1. Glu276 is pushed into the active site: Hydrophobic cavity is lost

2. H-bonding network (Gln136-Asp151-Arg156) is lost: Guanidinium group is not stable

Novel NA Inhibitors

1. Structural Modifications
2. Natural Products
3. Computer Simulations/ Virtual Screening
4. Novel NA Target
Structural Modifications

<table>
<thead>
<tr>
<th>Influenza Virus H1N1</th>
<th>A-315675 IC(_{50}) (nM) &amp; fold increase with WT</th>
<th>Tamiflu(^\circ) IC(_{50}) (nM) &amp; fold increase with WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.3 (+/-0.06)</td>
<td>1.74 (+/-0.01)</td>
</tr>
<tr>
<td>H274Y</td>
<td>5.5</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>R292K</td>
<td>7.0</td>
<td>&gt;3000</td>
</tr>
</tbody>
</table>

Alpinia katsumadai is a Medicinal plant used in traditional Chinese medicine
An antiviral herbal remedy

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ value (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.67 +/- 0.36</td>
</tr>
<tr>
<td>2</td>
<td>6.10 +/- 1.52</td>
</tr>
<tr>
<td>3</td>
<td>29.75 +/- 8.15</td>
</tr>
<tr>
<td>4</td>
<td>1.05 +/- 0.42</td>
</tr>
<tr>
<td>5</td>
<td>4.13 +/- 1.50</td>
</tr>
</tbody>
</table>

In vitro inhibition of NA of Influenza virus (H1N1) A/PR/8/34

Natural Products - Diarylheptanoids

NA Inhibitory Activity of Oseltamivir and 4 against Several Porcine H1N1 Isolates

<table>
<thead>
<tr>
<th>H1N1 Influenza Virus</th>
<th>Compnd 4 (µM)</th>
<th>Tamiflu (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Postdam/15/81</td>
<td>0.73</td>
<td>0.16</td>
</tr>
<tr>
<td>A/Belzig/2/01</td>
<td>0.59</td>
<td>0.20</td>
</tr>
<tr>
<td>A/Horneburg/IDT7489/08</td>
<td>1.11</td>
<td>0.06</td>
</tr>
<tr>
<td>A/Brest/IDT7490/08</td>
<td>1.64</td>
<td>0.19</td>
</tr>
</tbody>
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Structural Insights

Structural Insights

- Cation–π interactions; Arg152, Arg224, and Arg371
- H-bonding; cyclic ether and Arg292 and between the lactone and Glu119
- His274Tyr mutation; shift of Glu276 toward the binding pocket

Discovery of a Second Binding Site?

- N1 NA subtype - An additional site adjacent to the active site
- 150-loop region (147-152) (10 Å x 5 Å x 5 Å)
- Open conformation; preferred in group 1 NAs,
- Closed; preferred in group 2 NAs

H274Y mutation is specific to N1 subtype (H5N1, H1N1)

Key: design drugs with bulkier moieties to fit the adjacent cavity

A Novel Drug Binds to Cavity 150

- Synergistic effect with Tamiflu®
- Binds simultaneously with Tamiflu®

An J. et al., *Journal of Medicinal Chemistry* 2009, 52, 2667-2672
Possible Drug Candidates

An J. et al., Journal of Medicinal Chemistry 2009, 52, 2667-2672
Future Insights

RNA polymerase Inhibitors

Hemagglutinin Inhibitors

Small molecule inhibitors saponins with 3-O-β-chactriosyl

Song G. et al., J. Med. Chem. 2009, 52, 7368-7371

Promoter RNA binding site of viral RNA polymerase- A new target for drugs

Kuzuhara T. et al., JBC 2009, 284, 6855-6860

von Itzstein M., Nature Reviews Drug Discovery. 2007, 6, 967-974
Conclusion

- Pandemics of Influenza virus are global threats
- Emergence of resistance to common drugs has become critical health and economical problem
- Finding a novel effective drug against Influenza viruses has become an immediate global need
- Novel M2 ion channel inhibitors have not improved up to a satisfactory level
- Research on novel neuraminidase inhibitors have improved to a promising point after the discovery of novel drug binding site
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  - Danielle, Dennis, Dilini, Getrude, Irosha, Koyeli, Mark, Noelle, Ruth, Sean, Washington
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