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Management of Pandemic Influenza In ‘Have Not’ Countries

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The Pandemic Threat and Prospects for H5N1 Vaccination

- Inactivated adjuvanted vaccines
  - development slow, funding limited
  - production capacity limited - 9 countries
  - ~ 700 M people vaccinated with 2 doses in 6 months
  - stockpiling pre-pandemic vaccines possible in only a few countries
- Live-attenuated and recombinant HA vaccines
  - R&D slow and production problematic

Technical limitations are significant, but organizational, logistical and political limitations are even greater!


Tamiflu Treatment of Clade 2 H5N1 Influenza in Indonesia

<table>
<thead>
<tr>
<th>Interval between onset of illness and treatment</th>
<th>No. of cases</th>
<th>No. of deaths</th>
<th>Case fatality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 24 hours</td>
<td>2</td>
<td>0</td>
<td>0 %</td>
</tr>
<tr>
<td>0-4 days</td>
<td>11</td>
<td>5</td>
<td>45 %</td>
</tr>
<tr>
<td>0-6 days</td>
<td>37</td>
<td>24</td>
<td>65 %</td>
</tr>
<tr>
<td>&gt; 6 days</td>
<td>49</td>
<td>40</td>
<td>82 %</td>
</tr>
<tr>
<td>Any treatment</td>
<td>86</td>
<td>64</td>
<td>74 %</td>
</tr>
<tr>
<td><strong>NO TREATMENT</strong></td>
<td><strong>33</strong></td>
<td><strong>33</strong></td>
<td><strong>100 %</strong></td>
</tr>
<tr>
<td>All cases</td>
<td>119</td>
<td>97</td>
<td>82 %</td>
</tr>
</tbody>
</table>

ISRVI, Singapore, 3 March 2008

Confronting the Next Pandemic: Could We Use Something Other Than Vaccines and Antivirals?

- ↑↑ Pro-inflammatory cytokines in human macrophages and alveolar epithelial cells
- ↓ IFN-1 α(β) in bronchial epithelial cells


Hypercytokinemia in Fatal Cases of H5N1 Infection

- Fatal cases had higher levels of pro-inflammatory cytokines
  “Although immunomodulatory treatment has potential benefits at this stage, the focus of clinical management should be on preventing the intense cytokine response by early diagnosis and effective antiviral treatment.”
- All cases were diagnosed late; average 6 days (4-8)
- All but one of 18 cases were treated with antivirals, yet 13 (72%) died!
**Cell Signaling Pathways in Acute Lung Injury (ALI)**

- Inactivated H5N1 and H5N1 viruses instilled intratracheally in C57BL/6N mice
- Lung edema 5 hours later↑ only in H5N1-treated mice
- Inflammatory response to H5N1 confirmed histologically (NP and OxPLs) and by assays for ROS, NF\(\kappa\)B, IL-6


**Heme Oxygenase (HO)-1 Inhibits TLR4 Signaling by Regulating TLR Trafficking to Lipid Rafts**

- Ligand-mediated receptor trafficking to lipid rafts is an early event in signal initiation of immune cells
- LPS trafficking of TLR4 to lipid rafts is NADPH oxidase- and RAS-dependent
- ↑ HO-1 → ↑ CO → ↓ LPS-induced NADPH oxidase
- ↓ ROS → ↓ TLR4 translocation to lipid rafts
- ↓ TLR4 signaling → ↑ NF\(\kappa\)B activation
- ↓ pro-inflammatory cytokines


**Two Ways to Respond to the Pandemic Threat**

*Top-down approach - pandemic vaccines and antivirals*
- Involves only scientific, company and governmental elites
- Slow, complex and difficult to organize and manage

*Bottom-up approach*
- Uses ordinary people and existing health care systems
- Uses abundant supplies of inexpensive generic agents
- Would be available worldwide on the first day of a pandemic

Immunomodulatory Agents That Should Be Considered for Pandemic Use

- **Statins** – HMG-CoA inhibitors
  - Lower HDL cholesterol and prevent cardiovascular and cerebrovascular disease
  - Anti-inflammatory (pleiotropic) effects
- **PPARα agonists (fibrates)**
  - Regulate lipid metabolism, fatty acid oxidation
  - Anti-inflammatory and immunomodulatory effects
- **PPARγ agonists (glitazones)**
  - Increase sensitivity to insulin
  - Anti-inflammatory and immunomodulatory effects

* Peroxisome proliferator-activated receptor

Experimental Acute Lung Injury and the Effects of Statins and PPAR Agonists

<table>
<thead>
<tr>
<th>Cell signaling molecules</th>
<th>PPARα</th>
<th>PPARγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALI</td>
<td>Statins</td>
<td>Fibrates</td>
</tr>
<tr>
<td>TLR4</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>NFκB</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>ROS</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>HO-1 (↓)</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

Influenza Immunopathogenesis and the Effects of Statins and PPAR Agonists

<table>
<thead>
<tr>
<th>Cytokines/chemokines</th>
<th>Influenza</th>
<th>Statins</th>
<th>PPARα</th>
<th>PPARγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>IL-1</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>IFNγ</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>↑</td>
<td>↓</td>
<td>n.d.</td>
<td>↓</td>
</tr>
<tr>
<td>RANTES (CCL5)</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>IL-8</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>MIG</td>
<td>↑</td>
<td>↓</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>IP-10</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>


Resveratrol (RES) Improves Survival in PR8-infected Mice

- Balb/c mice treated 7d with RES
  - Virus replication ↓ 2 logs
  - Mortality ↓ 40%
  - No anti-oxidant effect (GSH)
  - Nuclear-cytoplasmic translocation of VNPs
  - Activity of cellular protein kinases (MAPK, JNK, not ERK 1/2)
  - Other non-antiviral effects


PPARα Agonist Gemfibrozil Improves Survival in Mice with H2N2 Influenza

- BALB/c mice - 50 controls, 46 treated with gemfibrozil 60 µg on days 4-10
- Mortality - controls 74% gemfibrozil 48%
- Hazard function 0.46 (95% CI 0.26-0.76)
- Gemfibrozil significantly reduced H2N2 mortality


Delayed Antiviral and Immunomodulatory Treatment Increases Survival in H5N1-infected Mice

- Balb/c mice → 1000 LD50
  - A/VN/1194/04 (H5N1)
  - Treatment (ip) @ 48 hx 6 d
  - Zanamivir
  - Z + celecoxib (COX-2 inhib)
  - Z + mesalazine (PPARγ agonist)
  - Z + C + M
  - No treatment
- Mortality
  - Z ↓ viral load but cytokines and mortality similar to no Rx
  - Z + C ↓ viral load similar to Z only, but ↓ inflammatory cytokines (p< 0.01) and ↓ mortality (p= 0.02)

COX-2-triggered Inflammatory Cascade in Humans and Mice

- In cell culture, H5N1 virus (and LPS) → ↑ COX-2 and inflammatory cytokines in macrophages but not in alveolar epithelial (A549) cells
- Supernatants from H5N1-infected macrophages → A549 cells → ↑ COX-2 and inflammatory cytokines
- COX-2 inhibitor treatment of macrophages exposed to H5N1 virus → no increase in COX-2 and cytokines in A549 cells

**Interpretation**

COX-2 maintains the pro-inflammatory cascade after a decrease in H5N1 replication via a "complex positive feedback loop" and may be a treatment target.


Epithelial Cell Inflammation in ALI is Followed by COX-2-dependent Active Resolution

- Early ALI - ↑ COX-2 → ↑ LT Ba, PTE, ROS, TNFα, ↑ NADPH oxidase, ↑ edema
- COX-2-derived PGD2 (LTB4) switches to PGE4 → ↑ lipoxins (LX), especially LXα
- LX - ↓ TNFα, IL-6 and ROS, ↓ pmn superoxide and chemotaxis, ↑ macrophage phagocytosis of apoptotic neutrophils, ↓ edema, ↓ VEGF-mediated angiogenesis, ↓ MMPs, ↑ HO-1
- Early COX-2 inhibition → ↓ pmn trafficking
- Late COX-2 inhibition → ↓ LXA4 → ↑ inflammation and prolonged recovery


Statins Activate PPARγ and PPARα Through COX-2 Expression in Macrophages

- Statins → ↑ ERK1/2 and p38 MAP kinase → ↑ COX-2 expression → ↑ prostaglandin 15d PGJ2 → ↑ PPARγ and PPARα
- Statins → ↓ TNFα and MCP-1 and these anti-inflammatory effects are inhibited by blocking PPARγ and PPARα


Because the anti-inflammatory effects of statins and PPARγ and PPARα agonists are mediated by COX-2, COX-2 inhibition might not always be helpful.

Mechanisms of Sepsis-induced Multi-organ Dysfunction


Statins, PPAR Agonists and Influenza Possible Mechanisms of Action

Non antiviral effects that improve cardiopulmonary endothelial and epithelial cell function and restore energy homeostasis in all vital organs

- ↓ NF-κB and ↓ AP-1 → ↓ cytokines, chemokines, cellular adhesion molecules; modify caspase activation and apoptosis
- ↑ pro-resolution lipoxin A4, resolvin D1, protectin D1
- ↑ eNOS → ↑ NO, ↑ vasodilatation, ↑ cardiovascular function
- Alter actin cytoskeleton and intracellular tight junctions, ↑ lung barrier function, ↓ vascular leak
- ↑ HO-1 → ↓ oxidative stress, ↓ TLR4 and downstream signaling
- Restore mitochondrial homeostasis, ↑ energy supply (ATP)
An Epidemiological ‘Signal of Protection’

Statins Reduced Pneumonia Mortality

- Population-based, nested case-control study of 1227 cases of pneumonia each matched with three controls
- Evaluated effects of statins prescribed during 30 days prior to hospitalization

<table>
<thead>
<tr>
<th>Statin reduction</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pneumonia hospitalization</td>
<td>0.63 (0.46 to 0.88)</td>
</tr>
<tr>
<td>30-day pneumonia mortality</td>
<td>0.47 (0.25 to 0.88)</td>
</tr>
</tbody>
</table>

Statins reduced pneumonia mortality by 53%


Statins Reduce ICU Pneumonia Mortality

Randomized Controlled Trial

- 67 ICU pneumonia patients randomized to receive atorvastatin 10 mg (33) or placebo (34) qd
- No differences between groups in age, cholesterol (day 1), APACHE II, ALI and SOFA scores and Pneumonia Severity Index
- Cholesterol lower in statin group on day 7
  - 90 vs. 118 mg/dl; p = 0.044

<table>
<thead>
<tr>
<th>% Mortality</th>
<th>Controls</th>
<th>Statin</th>
<th>% reduction</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU</td>
<td>50</td>
<td>27.3</td>
<td>45.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Hospital</td>
<td>55.9</td>
<td>27.3</td>
<td>51.2</td>
<td>0.026</td>
</tr>
</tbody>
</table>


Other Agents for Pandemic Treatment and Prophylaxis

- Consider any available and inexpensive generic anti-inflammatory, immunomodulatory or antiviral agent
  - COX-2 inhibitors
  - ACE inhibitors – ↓ ACE gene expression
  - angiotensin-II receptor blockers (ARBs)
  - spironolactone, phosphodiesterase inhibitors
  - resveratrol and chloroquine (both have anti-influenza activity)
  - naltrexone, metformin, ethyl pyruvate, curcumin → ↓ NF-kappa-B
  - bupropion → ↓ TNF-α, IFN-γ and IL-1β, ↑ IL-10
- If effective, these agents could be used alone, with antivirals, or as combination therapy with statins, PPAR agonists and/or other agents

Could Generic Agents Be Used for Pandemic Treatment and Prophylaxis?

- There is a global need for effective agents to complement limited supplies of vaccines and antivirals
  - Generic agents are now being produced in developing countries
    - simvastatin by ~ 102 companies, more than 50% located in China and India
    - fibrates by many companies, some in China and India
- Five-days of treatment in developing countries would be inexpensive
  - simvastatin → < $1
  - fibrates → ~ $3-5, probably much less


Five-point Research Agenda for Using Generic Agents in a Pandemic

1. Test candidate treatments in whole animals (mice, ferrets and non-human primates) to identify specific agents that might be effective in managing an H5N1-like pandemic
2. Later, study promising treatments in cell culture and animals to define the molecular mechanisms that explain their beneficial effects in H5N1 virus infection
   - molecular biologists who know cell signaling in inflammation, the immune response and energy homeostasis in acute lung injury, sepsis and multi-organ failure
   - laboratory investigators and clinicians from critical care, cardiovascular and pulmonary diseases, endocrinology and metabolism and pharmacology and therapeutics
3. Identify developing countries where these generic agents are produced, determine quantities produced, surge capacities, patterns of distribution and costs
4. Establish a process to manage the stockpiling of generic agents and/or their distribution in a pandemic
5. Conduct randomized controlled trials of promising treatments immediately after the emergence of a new pandemic virus

Fedson DS. The challenge of pandemic preparedness for developing countries: what’s missing. To be published.
Clinical Trial of a New Treatment Immediately After the Onset of the Next Pandemic

Total sample size (power)

<table>
<thead>
<tr>
<th>Mortality reduction</th>
<th>80%</th>
<th>90%</th>
<th>95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>530</td>
<td>690</td>
<td>850</td>
</tr>
<tr>
<td>50%</td>
<td>140</td>
<td>170</td>
<td>210</td>
</tr>
<tr>
<td>75%</td>
<td>60</td>
<td>80</td>
<td>90</td>
</tr>
</tbody>
</table>

Unpublished calculations assume 1:1 randomization of subjects to two treatment groups, untreated mortality = 50% and α = 0.05 (two-sided).

What Will Be The Consequences of the Next Pandemic?

How Will We Confront the Next Pandemic?

Generic agents might not work, but we have a choice

- We can undertake the necessary research before the pandemic arrives and show that generic agents will not be effective, or
- We can undertake the research after the pandemic has passed and show that millions could have been saved

Preparing for the Next Pandemic

Concluding Thoughts

- The next influenza pandemic might not bring on a global catastrophe, but everything we know about influenza virology tells us it could
- For the next five years and probably longer, a “top down” approach using pandemic vaccines and antivirals won’t meet the needs of > 85% of the world’s people who live in “have not” countries
- A “bottom up” approach the uses inexpensive and widely available generic agents could help meet their needs

For now, a “science for management” must supercede a “science of explanation”

Confronting the Next Pandemic With Inexpensive Generic Agents

“It is not enough to say, ‘We are doing our best.’ You have got to succeed in doing what is necessary.”

Winston Churchill

References


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