Ventilated Pig Models of Pseudomonas aeruginosa Pneumonia

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Presenter Disclosure Information

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1. NIH Development of the Mucus Slurper

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BACKGROUND AND RATIONALE FOR THE USE OF LARGE ANIMAL MODELS OF P. AERUGINOSA PNEUMONIA
“Clinical research must conform to the moral and scientific principles that justify medical research and should be based on laboratory and animal experiments or other scientifically established facts.”
Rationale for the use of pneumonia animal models

• To elucidate pathogenesis of the disease (in the absence of confounding factors)
• To characterize the role of bacterial and host factors in the disease
• To test efficacy and safety of novel preventive strategies and therapies
Small Animal Models of *P. aeruginosa* Pneumonia
**P. aeruginosa** Pneumonia Models in Small Animals

**Morphologic and Microbiologic Features of *P. aeruginosa* Pneumonia in normal Hamsters.** Coalson JJ, Higuchi JH, Williams ML, Johanson
*Exp and Mol Pathology* 1986, 45: 193-206


1974-2014 Original Articles on pneumonia in Mice, Rats and Rabbits

1974-2014 Original Articles on pneumonia in Pigs, Sheep, Dogs and Baboons

Source: MEDLINE
Mouse Models of *P. aeruginosa* Pneumonia: BENEFITS

- **Mouse:**
  - Smallest mammal
  - Rapid reproductive rate
  - Genetic and physiological similarities to humans
  - Potentials for extensive genome manipulations
  - Unique strains and genetically engineered mutants

- **Limited costs**
Mouse Models of *P. aeruginosa* Pneumonia: LIMITATIONS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mice Differ From Humans In:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomy of respiratory tract</td>
<td>1. Proportionally larger nasal surface area</td>
</tr>
<tr>
<td></td>
<td>2. Fewer, less symmetric airway branches</td>
</tr>
<tr>
<td></td>
<td>3. No respiratory bronchioles</td>
</tr>
<tr>
<td></td>
<td>4. Few mucous or serous cells</td>
</tr>
<tr>
<td></td>
<td>5. No submucosal glands below trachea</td>
</tr>
<tr>
<td>Physiology of respiratory tract</td>
<td>1. Obligate nose breathers</td>
</tr>
<tr>
<td></td>
<td>2. Weak cough reflex</td>
</tr>
<tr>
<td>Pattern recognition receptors</td>
<td>1. Cellular expression of TLRs 3, 5, and 9</td>
</tr>
<tr>
<td></td>
<td>2. Ligand specificity of TLRs 2, 4, and 9</td>
</tr>
<tr>
<td></td>
<td>3. Ligand specificity of Nod1</td>
</tr>
<tr>
<td>Antimicrobial secretions</td>
<td>1. Pattern of lysozyme secretion</td>
</tr>
<tr>
<td></td>
<td>2. Absence of hypothiocyanite</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>1. Lower circulating counts</td>
</tr>
<tr>
<td></td>
<td>2. No α-defensins</td>
</tr>
</tbody>
</table>

TLRs, Toll-like receptors; Nod1, human nucleotide binding and oligomerization domain protein 1.

Long term mechanical ventilation is unfeasible

Limited ability to provide critical care to severely ill animals in septic shock

As for VAP, primary pathogenic mechanism, through aspiration of oropharyngeal pathogens is difficult to reproduce

The ability of experiments in small animals to predict the long-term effectiveness and safety of therapies in humans remains controversial

ICU-related concomitant conditions difficult to replicate
• Age
• Coexisting Diseases
• Supportive therapies
• Severity of illness
• Advanced monitoring
Pseudomonas aeruginosa-Catecholamine Inotrope Interactions

A Contributory Factor in the Development of Ventilator-Associated Pneumonia?

Primrose P. Freestone, PhD; Robert A. Hirst, PhD; Sara M. Sandrini, PhD; Fathima Sharaff, MSc; Helen Fry, MD; Stefan Hyman; and Chris O’Callaghan, DM, PhD
Poor ability to use equipment and techniques developed for human applications
Duration of the Experiment

- Start Invasive MV
- 6h
- 12h
- 18h
- 24h
ASK THE EXPERTS
Are we beginning to believe that we can go so low in the mammalian species and still obtain valid information?

Would you as a critical care physician accept data from a drug study on an intensive care patient who was not only not resuscitated with fluid but who did not even have blood pressures and heart rates monitored?

Traber, Daniel L. PhD, FCCM
University of Texas Medical Branch (UTMB)
*Crit Care Med* 27(2), 1999, pp 255-256
It is rather unreasonable to believe that results from small-animal studies – started right after breakfast and completed in time for dinner – could be safely translated into valuable therapies for critically ill patients.

Theodor Kolobow, MD
Division of Pulmonary and Cardiac Assist Devices
National Institutes of Health, Bethesda, MD
VENTILATED ANIMAL MODELS OF
P. AERUGINOSA PNEUMONIA
Features To Carefully Consider

- Burden of bacterial inoculum
- *Via* of Inoculation
- Animal position during inoculation
- Bacterial strain
- Pathogen antimicrobials susceptibility
- Type of animals
- Length of mechanical ventilation
- Severity of the disease
$P.\text{aeruginosa}$ Strains and 24-h Mortality
*P. aeruginosa* Strains and 24-h Norepinephrine Requirements

![Graph showing norepinephrine dosage for different strains of *P. aeruginosa*]


Johanson’s Model: Methods

- Adult baboons (10-24 Kg)
- Tracheally intubated, anaesthetized (diazepam, ketamine) and paralyzed (pancuronium)
- Oleic acid (0.04 ml/Kg) infused into the right atrium
- Animals on mechanical ventilation up to 10 days
- Lateral supine position and turned every 2h
Oropharyngeal and Pulmonary Colonization

- Oropharyngeal flora similar to humans (α-hemolityc strep, Neisseria sp, Staph aureus)
- Pneumonia was confirmed at necropsy and through histology studies (bronchiole and alveolar space filling with PMN)
- Etiology identified through postmortem lung aspirate or ante mortem blood culture in the absence of another identifiable source of bacteremia

**Table 2**

Bacterial Causes of Primate Nosocomial Pneumonia

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>1</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
</tr>
<tr>
<td>P. aeruginosa and S. aureus</td>
<td>1</td>
</tr>
<tr>
<td>M. morganii and E. coli</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
</tr>
</tbody>
</table>
Johanson’s Model: Advantages and Limitations

- Closely reproduces human pathogenesis and etiology; yet, *P. aeruginosa* is not the only causative pathogen
- Pulmonary challenge highly variable
- High costs, labor intensive and time consuming (72 hours prior to VAP onset)
- Scarce animal availability
- Strict legislations, guidelines, and policies related to the care and use of research primates
Luna’s Model: Methods

- Large White-Landrace female piglets (20±2 kg)
- MV up to 72 hours: VT 15 mL/Kg; PEEP 0 cmH2O
- Sedation/Analgesia: Midazolam/Fentanyl Paralysis: Pancuronium bromide
- Hemodynamic monitoring: Arterial catheter into the femoral artery; Pulmonary catheter through the internal jugular vein
15 ml of a $10^6$ cfu/mL suspension of P. aeruginosa ATCC 27853 multi-susceptible inoculated into every lobe through a bronchoscope
Experimental Severe *Pseudomonas aeruginosa* Pneumonia and Antibiotic Therapy in Piglets Receiving Mechanical Ventilation

Carlos M. Luna, Sebastián Baquero, Sebastián Gando, Juan Risso Patrón, Joaquín García Morato, Oriol Sibila, Rubén Absi, Angela Famiglietti, Carlos A. Vay, Florencia Von Stecher, Carlos Agusti and Antoni Torres

*Chest* 2007;132:523-531
Microbiological/Histological Confirmation

• Upon autopsy, several specimens for bacteriologic and pathologic studies.
Luna’s Model: Advantages and Limitations

• Rapid development of severe pneumonia, within 24 h

• Ideal model to study efficacy and safety of new pharmacological and non-pharmacological therapies

• Extensive pulmonary bacterial challenge
New Animal Model of *P. aeruginosa* VAP

A Novel Porcine Model of Ventilator-associated Pneumonia Caused by Oropharyngeal Challenge with *Pseudomonas aeruginosa*

Gianluigi Li Bassi, M.D., Ph.D., Montserrat Rigol, D.V.M., Ph.D., Joan-Daniel Marti, R.P.T., Ph.D., Lina Saucedo, M.D., Otavio T. Ranzani, M.D., Ignasi Roca, Ph.D., Maria Cabanas, M.D., Laura Muñoz, B.Sc., Valeria Giunta, M.D., Nestor Luque, M.D., Mariano Rinaudo, M.D., Mariano Esperatti, M.D., Laia Fernandez-Barat, Ph.D., Miquel Ferrer, M.D., Ph.D., Jordi Vila, Ph.D., Jose Ramirez, M.D., Ph.D., Antoni Torres, M.D., Ph.D.

_Anesthesiology_ 2014; 120:1205-15
Our Goals

- To develop a novel model of *Pseudomonas aeruginosa* VAP to accurately reflect the key pathogenic mechanisms of the disease and most prevalent human etiology.
Methods

- Seven female Large White-Landrace pigs (Kg 30.5±1.8)
- Oro-Tracheal intubation (ETT n. 7.5 comprising HVLP cuff)
- MV up to 3 days: VT 10 mL/Kg; PEEP 0 cmH₂O
- Standard sedation, analgesia and paralysis
- Standard hemodynamic monitoring
Methods
Pigs were placed in the prone position, and the custom-made surgical bed, covered atop by an anti-slip fine ribbed rubber sheet, was oriented approximately 30 degrees in the anti-Trendelenburg position.
Bacterial Inoculum

• After 4 and 8 hours of MV:
  – 5 mL of $10^7$ cfu/mL culture of *PA 01 ATCC 27853* Ceftriaxone-resistant into the oropharynx

• Ceftriaxone to prevent colonization by endogenous pathogens
Autopsy
VAP Diagnosis

- Sampling was always performed in areas showing gross abnormalities, when present.
- VAP was confirmed according to a histological injury score $\geq 3$, associated with a quantitative $P. aeruginosa$ lobar culture $\geq 3$ log cfu/gr.
VAP was confirmed in 6 out of 7 pigs. In 55.56% of the cases, VAP developed in RML; in the RLL in 22.22%; in the LUL or LLL in 11.11%; and never in the RUL (p=0.032).
Characteristic histological pattern of median infection-related lung injury score

1
Purulent Mucus Plugging

4
Confluent Pneumonia

2
Bronchiolitis

3
Pneumonia

2
Bronchiolitis
Li Bassi’s Model: Advantages and Limitations

- Pulmonary aspiration of oropharyngeal secretions colonized by *P. aeruginosa*.
- Gravity-dependent dissemination of the infection
- VAP was associated neither with severe systemic clinical signs of infection nor with hemodynamic collapse.
- High Costs, labor intensive and time consuming (48-72 hours prior to VAP onset)
- Ideal to study pathophysiologic mechanisms of the disease, diagnostic strategies and to test effectiveness and safety of preventive strategies
APPLICATIONS
<table>
<thead>
<tr>
<th>Healthcare Industry</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayer, Leverkusen, Germany</td>
<td>THE EFFECTS OF AEROSOLIZED AMIKACIN IN PIGS WITH SEVERE PSEUDOMONAS AERUGINOSA PNEUMONIA</td>
</tr>
<tr>
<td>Toray Industries, Tokyo, Japan</td>
<td>EFFECTS OF HEMOPERFUSION WITH POLIMIXIN B-IMMOBILIZED FIBER COLUMN ON ENDOTOXIN CLEARANCE IN A MODEL OF SEVERE PSEUDOMONAS AERUGINOSA PNEUMONIA</td>
</tr>
<tr>
<td>Cardeas Pharma, Seattle, WA, USA</td>
<td>NEBULIZED FOSFOMYCIN AND AMIKACIN IN AN EXPERIMENTAL MODEL OF SEVERE PNEUMONIA INDUCED BY P. AERUGINOSA IN VENTILATED PIGS</td>
</tr>
<tr>
<td>Medimmune, Gaithersburg, MD, USA</td>
<td>EFFICACY OF MEDI3902 IN THE PREVENTION AND TREATMENT OF PSEUDOMONAS AERUGINOSA VENTILATOR-ASSOCIATED PNEUMONIA</td>
</tr>
<tr>
<td>Cubist, Lexington, MA, USA</td>
<td>THE EFFECTIVENESS OF CEFTOLOZANE/TAZOBACTAM IN COMPARISON WITH PIPERACILLIN/TAZOBACTAM IN AN EXPERIMENTAL MODEL OF SEVERE PSEUDOMONAS AERUGINOSA PNEUMONIA</td>
</tr>
</tbody>
</table>
Conclusions

• Animal models of pneumonia are essential tools:
  – To elucidate pathogenesis of lung infections
  – To characterize pathogen virulence factor, innate and adaptative immune response
  – To test efficacy and safety of novel therapies

• Several animal models of *P. aeruginosa* pneumonia represent milestones in this field and constitute the basis for future methodological improvements
Conclusions 2

• The pig model is the most appropriate model of *P. aeruginosa* pneumonia treated in the ICU (particularly VAP)
  – Close anatomical similarities with the human
  – Good survival, irrespective of the severity of the infection
  – Possibility of prolonged mechanical ventilation
  – Reproduction of the main pathogenic mechanisms of the human disease
Thank You

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