Respiratory Response to Toxic Injury (Lung)

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Safety Assessment
GlaxoSmithKline

27 July 2012

Species Differences*

<table>
<thead>
<tr>
<th>Species</th>
<th>Lung volume</th>
<th>ELF volume</th>
<th>Number of alveoli</th>
<th>Alveolar diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>30.8 ml</td>
<td>200 µl</td>
<td>19.7 x 10^6</td>
<td>70 µM</td>
</tr>
<tr>
<td>Dog</td>
<td>1322 ml</td>
<td>14.6 ml</td>
<td>1040 x 10^6</td>
<td>126 µM</td>
</tr>
<tr>
<td>Human</td>
<td>4341 ml</td>
<td>24 ml</td>
<td>486 x 10^6</td>
<td>219 µM</td>
</tr>
</tbody>
</table>

Structure* and Function*: architecture, branching, physiology
Inhalation
Gas exchange

Histology and response to injury*: cell types and distribution, vulnerability, clearance, inflammation, background changes
Respiratory Response to Toxic Injury (Lung)

<table>
<thead>
<tr>
<th>Orders of branching</th>
<th>Length (mm)</th>
<th>Volume (ml)</th>
<th>Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>220</td>
<td>80</td>
<td>2.0</td>
</tr>
<tr>
<td>14</td>
<td>140</td>
<td>71</td>
<td>13.9</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>44</td>
<td>70.8</td>
</tr>
<tr>
<td>9</td>
<td>3.8</td>
<td>805</td>
<td>781.0</td>
</tr>
<tr>
<td>3-9</td>
<td>2.6</td>
<td>2000</td>
<td>7x10⁵</td>
</tr>
</tbody>
</table>

Tidal Volume (ml) | Minute Ventilation (ml/min)
Rat 2 | 150
Dog 200 | 3100
Monkey 20 | 750
Human 500 | 7000
Respiratory Response to Toxic Injury (Lung)

Bronchial Cell types

<table>
<thead>
<tr>
<th>Airway Type</th>
<th>Lining Cell</th>
<th>Secretory Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchi</td>
<td>Ciliated cell</td>
<td>Goblet cell</td>
</tr>
<tr>
<td>Terminal bronchiole</td>
<td>Ciliated cell</td>
<td>Clara cell</td>
</tr>
<tr>
<td>Bronchiole</td>
<td>Type I</td>
<td>Type II</td>
</tr>
</tbody>
</table>

Respiratory Response to Toxic Injury (Lung)

Epithelia of conducting airways

Species Differences

<table>
<thead>
<tr>
<th>Airway</th>
<th>Species</th>
<th>Cell types (approx %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>20</td>
<td>30 0 40 &lt;1</td>
</tr>
<tr>
<td>Mouse</td>
<td>10</td>
<td>40 50 0 &lt;1</td>
</tr>
<tr>
<td>Bronchi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>20</td>
<td>40 0 20 &lt;1</td>
</tr>
<tr>
<td>Mouse</td>
<td>2</td>
<td>40 55 0 &lt;1</td>
</tr>
<tr>
<td>Bronchiole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>0</td>
<td>60 40 0 0</td>
</tr>
<tr>
<td>Mouse</td>
<td>0</td>
<td>40 60 0 0</td>
</tr>
</tbody>
</table>

*other cells e.g., neurosecretory, eccrine also present

Respiratory Response to Toxic Injury (Lung)

Mucociliary clearance

- Defense against insoluble particulates
- Ciliated cells - co-ordinated movement
- Secreting cells - mucus, serous
Respiratory Response to Toxic Injury (Lung)

- 2 layers of fluid
  - sol - lower level, watery allows cilia to move
  - gel - upper layer of mucus

- Lining fluid contains secretions - IgA, lysozyme, anti-proteases
  - SLPI, elafin (elastase-specific inhibitor), cytokines
Respiratory Response to Toxic Injury (Lung)
Response to Toxic Injury – Airway epithelium (carina)

Respiratory Response to Toxic Injury (Lung)
Response to Toxic Injury – Airway epithelium (carina)

Respiratory Response to Toxic Injury (Lung)
Diffuse large airway disease

Resistance through a tube is proportional to $\frac{1}{(\text{radius})^{2}}$
**Respiratory Response to Toxic Injury - Airways**

<table>
<thead>
<tr>
<th>Airway Type</th>
<th>Lining Cell</th>
<th>Secretary Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory/ Terminal bronchiole</td>
<td>Ciliated cell</td>
<td>Clara cell</td>
</tr>
<tr>
<td>Alveoli</td>
<td>Type I</td>
<td></td>
</tr>
</tbody>
</table>

**Small Airways as Critical Sites for Damage**

- Particle size of most microorganisms and irritant dusts (0.5-2.0 μm) effectively reach the bronchoalveolar junction (BAD) level and deposit
- No protective mucus layer (due to gas exchange function)
- Absence of ciliary apparatus

**Mucus Thickness**

**Pollutant Concentration**

**Cell Death**
Respiratory Response to Toxic Injury - Small Airways

Bronchioalveolar duct junction (BADJ) common site for pathology
Respiratory Response to Toxic Injury - Alveoli

Terminal Airflow in the airspaces

Respiratory Response to Toxic Injury - Alveoli

Alveolar Capillaries

Respiratory Response to Toxic Injury - Alveoli

Alveolar Cell Types

<table>
<thead>
<tr>
<th>Airways Type</th>
<th>Lining Cell</th>
<th>Secretory Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchiole</td>
<td>Clara cell</td>
<td>Clara cell</td>
</tr>
<tr>
<td>Terminal bronchio</td>
<td>Clara cell</td>
<td>Clara cell</td>
</tr>
<tr>
<td>Alveolus</td>
<td>Type I</td>
<td>Type II</td>
</tr>
</tbody>
</table>
Respiratory Response to Toxic Injury - Alveoli

Alveolar epithelium

- Type I cells -
  - squamous, large surface area - susceptible to damage
  - maintain barrier to fluid loss, allow diffusion of gases,
  - cannot divide
- Type II cells -
  - cuboidal, lamellar bodies (surfactant), corners of alveoli
  - divide to replace type I and II cells
  - synthesizes surfactant, type IV collagen, PAF,
  - aa metabolites
  - modulate macrophage function

Respiratory Response to Toxic Injury – Alveoli

Retention and Clearance of Deposited Particles

Alveolar Region:
- No protective mucous layer due to gas exchange function
- Soluble particles
  - Pass through membrane
    - into blood
- Insoluble particles
  - Engulfed by alveolar macrophages
    - lymph nodes
    - Mucociliary escalator

Respiratory Response to Toxic Injury – Alveoli

Alveolar macrophages
Respiratory Response to Toxic Injury – Airspaces (Alveoli)
Retention and Clearance of Deposited Particles
Macrophage accumulation

Respiratory Response to Toxic Injury – Alveoli
Alveolar inflammation

Respiratory Response to Toxic Injury – Alveoli
Alveolar edema
Respiratory Response to Toxic Injury – Alveoli

Diffuse alveolar damage
Respiratory Response to Toxic Injury – Alveoli

Alveolar destruction - emphysema

Response to Foreign Material
Respiratory Response to Toxic Injury – Alveoli

Inhaled inert material

Respiratory Response to Toxic Injury – Alveoli

Alveolar fibrosis (Silica – 30 days)
Respiratory Response to Toxic Injury

Collection / Inflation Procedures for Lung

I. Syringe technique

II. Constant pressure technique

Importance of Fixation

Syringe technique
Respiratory Response to Toxic Injury

Constant pressure technique

- Advantages
  - Reasonably inexpensive
  - Excellent control over pressure
  - Least amount of artifact

- Disadvantages
  - Requires more time
  - Availability of apparatus

Airway Macrophages

Respiratory Response to Toxic Injury (Lung)

Case Study

Macrophage aggregations in the lung

Adverse? Non-adverse? Incidental?
Variable terminology for macrophages in the lung

- alveolar foamy macrophages
- alveolar histiocytosis
- prominent alveolar macrophages
- intra-alveolar macrophages
- foamy alveolar macrophages
- increased macrophage aggregates
- alveolar macrophage aggregation
- macrophage aggregation, alveolar

Alveolar macrophages

Rat 4 Week Tox Study

Female High Dose
Respiratory Response to Toxic Injury – Alveoli

Rat 4 Week Tox Study

Foamy Macrophages (2)

- Rat 26-week study (30 and 300 mg/kg/day), alveolar foamy macrophages in control rats (7/12 control males & 5/12 control females) in rats at the end of treatment period
- In males, foamy macrophages similar to concurrent controls, but at slight grade in a small proportion of animals with no clear dose response
- In females, an increased alveolar foamy macrophages in all treated groups. Some rats at slight grade & single rat at 300 mg/kg/day at moderate grade
- Recovery group: increased incidence in males & an increase in severity in females at 300 mg/kg/day at end of recovery phase

End of dosing phase:

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>300</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

End of recovery phase:

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>300</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Rat Foamy Macrophages – Historical Data
Incidence and Severity of Foamy Alveolar Macrophages in Lung from a Carcinogenicity Study in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>L</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>LI</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HI</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
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Respiratory Response to Toxic Injury – Alveoli

Alveolar macrophage response

What is clinical & regulatory significance of foamy macrophages in lungs from oral tox studies in rat (4 and 26 wk)?

“Prior to long term clinical trials, provide adequate justification that the finding is within spontaneous background and/or is not an adverse finding, or identify a NOAEL dose in the rat by conducting an additional toxicity study”.

Respiratory Response to Toxic Injury – Alveoli

Unique aspects of Respiratory System

• Tremendous surface area
• Highly elastic tissue
• Low tissue density
• Delicate fluid air interface
• Dual blood supply
• Multiple types of air conducting units
• Complexities of cell types and locations
• Species variations

• Back ups
### Differences - rodent and human

<table>
<thead>
<tr>
<th>Anatomical features</th>
<th>Human</th>
<th>Rodent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleura</td>
<td>Thick</td>
<td>Thin</td>
</tr>
<tr>
<td>Airway Size</td>
<td>Small calibre</td>
<td>Large calibre</td>
</tr>
<tr>
<td>Submucosal glands</td>
<td>Abundant</td>
<td>Rare</td>
</tr>
<tr>
<td>Respiratory bronchioles</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Clara cell</td>
<td>Low numbers</td>
<td>High numbers</td>
</tr>
<tr>
<td>Mucus cell</td>
<td>Abundant</td>
<td>Rare (serous)</td>
</tr>
<tr>
<td>Cardiac muscle in pulmonary veins</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>
Inflation Fixation

• Syringe technique
  – Advantages
    • Quick, Inexpensive, Practical, Convenient
  – Disadvantages
    • Risk of variation in pressure
    • Risk of overinflation, alveolar rupture