Abstract

Determination of the 50% Lethal Dose (LD₅₀) of Bacillus anthracis in Mice Following Inhalation Exposure

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Abstract

Background: The 50% lethal dose of Bacillus anthracis Ames was determined in mice. Eight groups of mice, each containing 10 BALB/c, were exposed to USP Water Vapor for Inhalation or Bacillus anthracis Ames by nose-only inhalation.

Methods: Various aerosol concentrations were delivered in the breathing zone of the mice corresponding to inhalation doses of 0 CFU (Group 1), 3.125 × 10⁴ CFU (Group 2), 6.25 × 10⁴ CFU (Group 3), 5.0 × 10⁵ CFU (Group 4), 1.0 × 10⁶ CFU (Group 5), 5.0 × 10⁶ CFU (Group 6), 6.25 × 10⁶ CFU (Group 7), 1.0 × 10⁷ CFU (Group 8). All mice were exposed for 30 minutes. These were suspended using a primary flow calibration device (100% dilution, 3.125 × 10⁴ CFU, 3.125 × 10⁵ CFU). Afterwards, the mice were monitored in a climate-controlled chamber until they were humanely euthanized. The exposure mixture contained 2% cotton and 4% water. The chamber was pressurized at 150 mm Hg to maintain an aerosol concentration of 250 ± 50 CFU/cm³.

Results

Methods Continued

Inhalation Exposure System

A Collison three-jet nebulizer (RCK Inc., Wallula, WA) was used. A single dose of 10 mg/mL trimethoprim and 10 mg/mL sulfamethoxazole was administered intraperitoneally to all mice, except for the control group, which received an equivalent volume of saline. After the inhalation exposure, the mice were monitored for 2 hours. The exposure mixture contained 2% cotton and 4% water. The chamber was pressurized at 150 mm Hg to maintain an aerosol concentration of 250 ± 50 CFU/cm³.

Results Continued

Aerosol Concentration

Liquid impinger samples were collected from the exposure plenum during each exposure. Inhalation plume counts were used to calculate aerosol concentration. Gayton's formula (1) was used to estimate mouse nostrils ventilation. Aerosol concentration, mouse nostrils ventilation, and exposure time were used to calculate inhalation dose. Group mean aerosol concentrations and mean inhalation doses are given in Table 2.

Table 2: Mean Aerosol Concentration and Mean Inhalation Dose

<table>
<thead>
<tr>
<th>Group</th>
<th>Aerosol Concentration (CFU/mL)</th>
<th>Inhalation Dose (mg)</th>
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<tbody>
<tr>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>1.25 × 10⁴</td>
<td>1.25 × 10⁴</td>
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<tr>
<td>3</td>
<td>2.50 × 10⁴</td>
<td>2.50 × 10⁴</td>
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<tr>
<td>4</td>
<td>5.00 × 10⁴</td>
<td>5.00 × 10⁴</td>
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<tr>
<td>5</td>
<td>1.0 × 10⁵</td>
<td>1.0 × 10⁵</td>
</tr>
<tr>
<td>6</td>
<td>2.50 × 10⁵</td>
<td>2.50 × 10⁵</td>
</tr>
<tr>
<td>7</td>
<td>5.00 × 10⁵</td>
<td>5.00 × 10⁵</td>
</tr>
<tr>
<td>8</td>
<td>1.0 × 10⁶</td>
<td>1.0 × 10⁶</td>
</tr>
</tbody>
</table>

Discussion

Inhalation aerosols in the most severe form of disease and the most rapid onset. Once inhaled, Bacillus anthracis Ames (BAA) spores germinate and release toxic factors which cause internal bleeding, swelling, and tissue necrosis. There are usually two stages of inhalation stage. Stage one can last from hours to days and presents with flu-like symptoms such as fever, fatigue, and malaise. Stage two usually develops slowly. Symptoms include (avers, shortness of breath, and shock). Male and female mice were exposed to concentrations of Bacillus anthracis Ames spores ranging from 10⁷-10⁸ CFU/mL in a nose-only inhalation exposure system. The 50% lethal dose was projected to be 1.0 × 10⁶ CFU/mL for male and female mice collectively. Separate analyses for male mice and female mice projected 50% lethal doses of 7.0 × 10⁵ CFU/mL for male mice, 3.1 × 10⁶ CFU/mL for female mice. Male and female mice were exposed to concentrations of Bacillus anthracis Ames spores ranging from 10⁷-10⁸ CFU/mL in a nose-only inhalation exposure system. The 50% lethal dose was projected to be 1.0 × 10⁶ CFU/mL for male and female mice collectively. Separate analyses for male mice and female mice projected 50% lethal doses of 7.0 × 10⁵ CFU/mL for male mice, 3.1 × 10⁶ CFU/mL for female mice. Male and female mice were exposed to concentrations of Bacillus anthracis Ames spores ranging from 10⁷-10⁸ CFU/mL in a nose-only inhalation exposure system. The 50% lethal dose was projected to be 1.0 × 10⁶ CFU/mL for male and female mice collectively. Separate analyses for male mice and female mice projected 50% lethal doses of 7.0 × 10⁵ CFU/mL for male mice, 3.1 × 10⁶ CFU/mL for female mice. The results of this study suggest a more rapid inhalation concentration of 50% lethal dose in BALB/c mice following nose-only inhalation exposure of Bacillus anthracis Ames spores of 9.4 × 10⁵ CFU/mL.

Conclusions

• Impaired analysis demonstrated that the actual aerosol concentration of the BAA spores was similar to the projected targets.

• Aerosol particle size analysis demonstrated the particle size distribution was similar for all groups.

• Protein analysis shows that BALB/c mice and female mice combined BALB/c mice following nose-only inhalation exposure of was 9.4 × 10⁵ CFU/mL.

References


Acknowledgements

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