Preclinical Experiments Support Recent Clinical Switch to Delivered Dose During Methacholine Bronchoprovocation Testing

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Background & Objectives

• Airway hyperresponsiveness (AHR) testing is a clinically used, central element of asthma diagnostics.
• The methacholine concentration of inhaled aerosol causing a 20% decrease in forced expiratory volume in 1 second (PC20) has typically been used to quantify the degree of AHR.
• The most recent standard of the European Respiratory Society (ERS) - endorsed by the American Thoracic Society (ATS) - recommends the effective delivered dose of methacholine (PD20), rather than concentration (PC20) (Coates et al., 2017)
• Expectation: PD20 (not PC20) allows for comparison of AHR results from different aerosol devices or protocols.

Objectives:

» Assess the validity of this expectation in mouse experiments
» Evaluate the use of the lung-deposited dose

Methods

• Healthy C57BL/6 mice (age:8-14 w); male (27-33g), female (19-22g)
• Anaesthesia, oral intubation, mechanical ventilation (flexiVent; emka TECHNOLOGIES-SCIREQ Inc.)
• AHR assessments to increasing concentrations of aerosolized methacholine (0 – 25 mg/mL)
  » At different times: between 2013 – 2017
  » In two different laboratories: CPC, RBC
• Two aerosol delivery protocols: EMKA (standard) and HMGU (maximized delivery)
  » Changes in respiratory resistance: forced oscillation technique (single frequency, 2.5Hz)
  » Delivered (inhaled) aerosol dose: gravimetric determination
  » Lung-deposited dose: concentration of a fluorescent tracer in lung homogenates

Experimental Set-up

Aerosol Delivery Protocols

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EMKA (standard)</th>
<th>HMGU (maximized delivery)</th>
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</thead>
<tbody>
<tr>
<td>Respiratory frequency (bpm)</td>
<td>150 bpm</td>
<td>120 bpm</td>
</tr>
<tr>
<td>Tidal Volume</td>
<td>10 mL/kg</td>
<td>400 μL</td>
</tr>
<tr>
<td>Inspiratory/expiratory ratio</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>PEEP (cmH2O)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Nebulizer ID</td>
<td>Aeroneb Pro#204</td>
<td>Aeroneb Lab#093</td>
</tr>
<tr>
<td>Droplet volume median diameter (μm)</td>
<td>4.0 – 6.0</td>
<td>3.5 – 4.0</td>
</tr>
<tr>
<td>Output rate – continuous (μL/min)</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>Nebulizer duty cycle (%)</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Nebulizer on-time/breath (ms)</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Nebulized volume (μL)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Nebulization time/challenge (s)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mass median droplet diameter (μm)</td>
<td>5.07</td>
<td>3.94</td>
</tr>
<tr>
<td>Delivery efficiency – dose delivered: loaded dose ratio (%)</td>
<td>8.4 ± 0.7</td>
<td>40.4 ± 1.0</td>
</tr>
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Delivered Aerosol Fraction

HMGU protocol: 0.404
EMKA protocol: 0.084

Delivered Dose Fraction

Deposited Dose Fraction

Conclusions

• Variations in aerosol delivery protocol affect the dose (delivered & deposited) of methacholine in mice.
• As expected, expressing airway hyperresponsiveness (AHR) results in terms of dose allows direct study comparisons between laboratories employing different equipment and aerosol delivery protocols.
• The present results thus support the updated clinical ERS standard recommending the dose – rather than concentration - as measure for bronchial methacholine challenge testing.
• While the delivered dose helps to attenuate protocol-specific differences, the lung-deposited dose could represent a better metrics for measuring AHR, especially when adjusted to the subject’s weight.
• As clinically recommended, the dose of methacholine (delivered or deposited) should be used to standardize preclinical (and clinical) AHR testing.

References: