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

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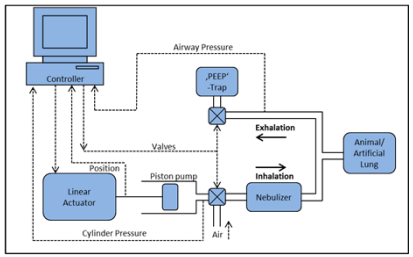
Background & Objectives

- 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



System for intubated ventilated aerosol delivery (methacholine) and lung function measurement in mice (flexiVent, EMKA)

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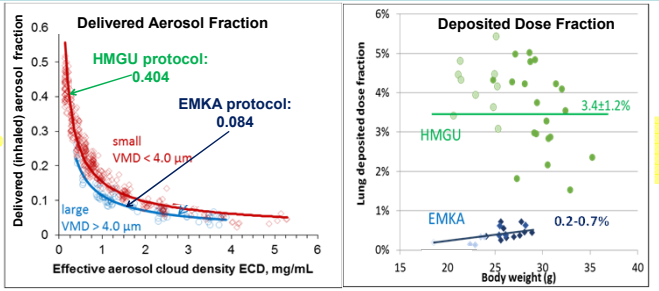
Aerosol Delivery Protocols

Parameters	EMKA (standard)	HMGU (maximized delivery)
Respiratory frequency (bpm)	150 bpm	120 bpm
Tidal Volume	10 mL/kg	400 µL
Inspiratory:expiratory ratio	2:3	2:1
PEEP (cmH_2O)	3	3
Nebulizer ID	Aeroneb Pro/#204	Aeroneb Lab/#093
Droplet volume median diameter (µm)	4.0 – 6.0	3.5 - 4.0
Output rate – continuous (µL/min)	500	250
Nebulizer duty cycle (%)	50	100
Nebulizer on-time/breath (ms)	80	20
Nebulized volume (µL)	10	10
Nebulization time/challenge (s)	10	70
Mass median droplet diameter (µm)	5.07	3.94
Delivery efficiency – dose delivered: loaded dose ratio (%)	8.4 ± 0.7	40.4 ± 1.0

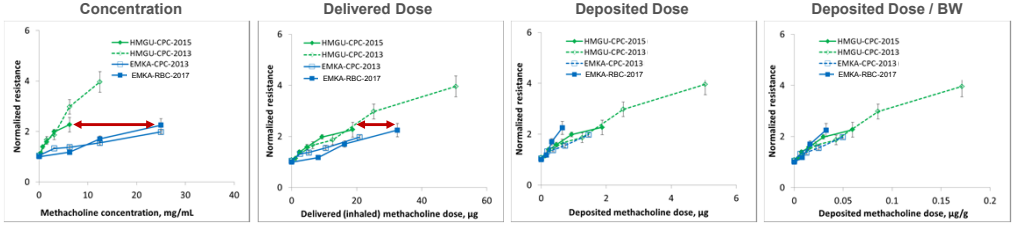
Results

10 µl of methacholine nebulized



Methacholine volume reaching the lungs:
HMGU : 0.34 µl
EMKA: 0.02 – 0.07 µl

Methacholine Airway Responsiveness (AHR)



Attenuation of protocol-specific differences

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:

Robichaud, A., Fereydoonzad, L. and Schuessler, T. F. (2015). Delivered dose estimate to standardize airway hyperresponsiveness assessment in mice. *Am J Physiol Lung Cell Mol Physiol* 308, L837-L846.

Coates, A. L., S. D. Dell, D. W. Cockcroft, and G. M. Gauvreau. (2017). The PD20 but not the PC20 in a methacholine challenge test is device independent. *Ann Allergy Asthma Immunol* 118 (4):508-50

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