Nebulization Patterns Affect Airway Hyperresponsiveness in Mice

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» Abstract

Rationale: The objective of this study was to assess how factors other than drug concentration affect the bronchoconstriction of mouse lungs to inhaled methacholine (MCh).
Methods: Anaesthetized, tracheostomized A/J mice were connected to a SCIREQ *flexiVent* and subjected to increasing concentrations of aerosolized MCh. Aerosols were generated using computer-controlled Aeroneb nebulizers with varied nebulization times (2.5-20s), rates (25-100%) or particle size spectra (2.5-4 vs 4-6 um). The effect of a drying tube on the air intake was also evaluated. Bronchoconstriction was assessed pre- and post-challenge using single frequency and broadband forced oscillations (FO) measurements of respiratory mechanics, and peak responses of all FO parameters (R, E, R_N, G, H) were recorded.
Results: FO parameters consistently increased as MCh concentration rose regardless of the condition. MCh-induced bronchoconstriction was not affected by changes in the nebulization time as evidenced by superimposed dose-response curves of all FO parameters. A higher nebulization rate reduced responsiveness, significantly augmenting the MCh dose required to induce a six-fold (PD600) increase in E (25%: 262 ± 105 vs 100%: 848 ± 83 ug) and G (25%: 223 ± 70 vs 100%: 724 ± 30 ug) or four-fold (PD400) in H (25%: 320 ± 120 vs 100%: 1004 ± 132 ug). Reducing the particle size range did not significantly affect PD400 for all FO parameters when both nebulizers were used at matched output rate. FO parameters were also not affected by the presence or absence of a drying tube (at 45% ambient humidity).
Conclusion: Results demonstrate that all aspects of the nebulization process must be considered when designing inhaled protocols. MCh responsiveness in mice was inversely related to the nebulization rate, an effect presumably linked to particle agglomeration at higher rates. The study provides means of refining airway hyperresponsiveness studies and confers practical implications for researchers.

» Background

The physiological response to an inhaled bronchoconstrictor challenge can potentially be influenced by various factors such as:

- the drug concentration
- the type of nebulizer
- the duration and rate of nebulization
- the disease state and severity

» Objectives

Evaluate the effect of factors other than drug concentration on the physiological response to an aerosolized bronchoconstrictor challenge in mice.

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» Methods

Protocol

- Male A/J mice
- Anesthetized, tracheostomized
- Connected to *flexiVent* for mechanical ventilation
  - 150 breath/min, 10 mL/kg, PEEP = 3 cm H_2O
- Aerosolized methacholine (Mch)
  - Aeroneb nebulizers
  - Gravimetric characterization of nebulizer outputs
  - Synchronized with inspiration
  - MCh dose calculations

Experimental Conditions

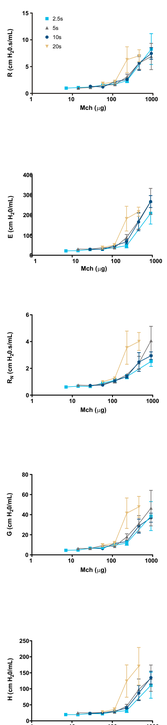
- Nebulizing time: 2.5-20 s
- Nebulizer rate (duty cycle): 25-100%
- Nebulizer type:
  - Standard (4-6 µm)
  - Fine Mist (2.5-4 µm)
- Humidity:
  - Presence of drying tube on air intake
  - Absence of drying tube on air intake

Lung Function Measurements

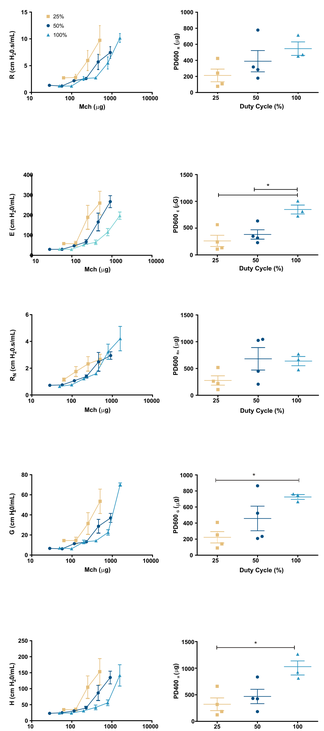
- *SnapShot-150, Quick Prime 3*
- At baseline
- After MCh challenge (0-200 mg/mL)
  - 12 measurements
  - Every 10 s
  - Alternating perturbations
- Between MCh challenges
- Parameters: R, E, R_N, G, H

» Results

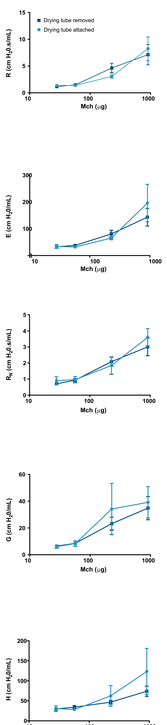
Nebulization Time
(Standard nebulizer, 50% duty cycle, n=4-8)



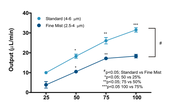
Nebulizer Rate (Duty Cycle)
(Standard nebulizer, 10s)



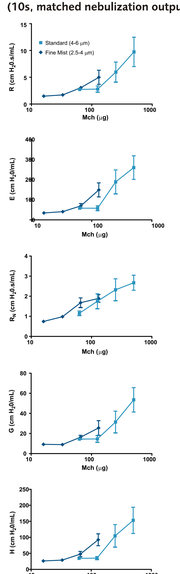
Humidity Control
(Standard nebulizer, 50% duty cycle, 10s, 45% ambient humidity, n=3)



Nebulizer Characterization



Nebulizer Particle Size
(10s, matched nebulization output rates, n=4-8)



» Summary

- FOT parameters augmented with increasing MCh.
- MCh-induced bronchoconstriction was independent of:
  - Nebulization time
  - Nebulizer particle size
  - Humidity control (45% relative humidity)
- Inversely related to nebulizer duty cycle

» Conclusions

- All aspects of the nebulization process must be considered when designing inhaled bronchoconstrictor protocols.
- The nebulizer duty cycle influenced the sensitivity of the physiological response to MCh.
- Effect presumably associated with particle agglomeration.
- Optimized physiological lung responses to an aerosol challenge can be obtained by adequate control over the nebulizer settings.

Disclosure

AR, LGG, PL & TFS are employed by SCIREQ Scientific Respiratory Equipment Inc., a commercial entity involved in a subject area related to the content of this presentation. TFS also owns stock.

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