An effective inhalation system for preclinical pulmonary drug delivery in mice

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Chronic lung diseases such as chronic obstructive pulmonary disease (COPD), lung cancer, and asthma are the second leading cause of death worldwide (WHO). Currently, there is no causal cure for any of these diseases. Hence, research in this field is expected to intensify significantly in the upcoming years. In addition, recent technological advances in inhalation technology have provided efficient, breath-controlled inhalation devices which are making inhaled drugs increasingly more attractive not only for the treatment of lung disease, but also for non-invasive systemic drug delivery. Preclinical development of new drugs and treatment strategies for inhalation therapy requires efficient, dose-controlled, aerosolized drug delivery systems for animal models. However, the preclinical development of aerosolized drugs for inhalation therapy is hampered by the lack of easy-to-use and yet efficient *in vivo* aerosol inhalation devices for preclinical animal models.

Especially for mice, the currently available methods for pulmonary drug application are either inefficient or not using (clinically relevant) nebulizers. In this study a commercially available ventilation apparatus for lung function measurement (Flexivent, Scireq Inc., Canada) was investigated for dose-controlled aerosol delivery to the lungs of intratracheally intubated and mechanically ventilated mice (intubated-ventilated mice). For the first time this would combine diagnostics of the disease state (lung function testing) and aerosolized pulmonary drug delivery for a given mouse using the same device.

The systematic experimental investigation of the effects of seven different operational parameters (various ventilation parameters, tubing length and various nebulizer properties such as liquid output rate and droplet size) on the aerosol dose delivered to the intubation cannula revealed a wide range of aerosol delivery efficiencies ranging from about 5% to 50% (of invested amount of liquid) depending on operational parameters. Our study also showed that the combined effects of the seven different operational parameters on cannula-delivered aerosol dose can be described by a single characteristic parameter, the effective aerosol density EAD (Figure 1). Hence, EAD allows both prediction of the cannula-delivered aerosol dose (proportional to $EAD^{-2/3}$) and selection of the best operational parameters for most efficient pulmonary drug delivery with the Flexivent system.

As proof-of-concept study aerosolized methacholine (induces bronchoconstriction) was delivered to C57BL/6 mice with the Flexivent system and the measured airway hyperresponsiveness (lung function) for two different methacholine delivery protocols (standard and dose-optimized). Taking the differences in cannula-delivered dose into account both delivery protocols resulted in the same changes in lung function parameters. This confirms that cannula-delivered aerosol dose corresponds with biological response and hence lung-delivered aerosol dose. Moreover, this also implies that the newly defined EAD parameter may be a useful tool for assessing the cannula-delivered (lung-delivered) aerosol dose for "arbitrary" operational settings of the Flexivent system and thus inter-comparison of hyperresponsiveness data obtained with different methacholine nebulization protocols is possible.

For the first time the pulmonary aerosol dose to the subject (mouse) can be estimated based on nebulizer characteristics, device and ventilation parameters, making comparison of different studies and different devices possible.

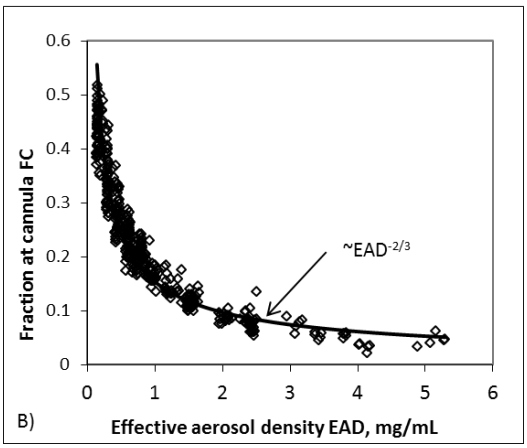

Figure 1. Fraction of invested liquid reaching the intubation cannula (FC) versus effective aerosol density (EAD). FC correlates with EAD according to $\sim EAD^{-2/3}$ ($p < 0.001$).

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