Journal of Pharmacological and Toxicological Methods 114 (2022) 107156

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Intratracheal administration of solutions in mice; development and validation of an optimized method with improved efficacy, reproducibility and accuracy

Charlotte E. Pelgrim [a,*], Ingrid van Ark [a], Thea Leusink-Muis [a], Maike A.D. Brans [b], Saskia Braber [a], Johan Garssen [a,c], Ardy van Helvoort [d,e], Aletta D. Kraneveld [a], Gert Folkerts [a]

[a] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, the Netherlands
[b] Experimental Cardiology, University Medical Center Utrecht, the Netherlands
[c] Platform Immunology, Danone Nutricia Research, Utrecht, the Netherlands
[d] Danone Nutricia Research, Utrecht, the Netherlands
[e] NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Department of Respiratory Medicine, Maastricht, the Netherlands

ARTICLE INFO

Keywords:
Instillation
Intratracheal
Intubation
Methods
Oropharyngeal

ABSTRACT

Animal models are still vital in the field of respiratory disease research. To improve the accuracy and consistency of the dose of specific compounds administered specifically in the respiratory tract, it is important to optimize and to compare the technique to currently available techniques. In this study, an optimized intubation-mediated intratracheal administration (IMIT) technique is described and compared to oropharyngeal aspiration (OA).

Adult female Balb/c mice were treated with Evans Blue using IMIT or OA and sacrificed after a short recovery to observe the distribution of solutions throughout the lungs. Additionally, mice were treated with increasing doses of lipopolysaccharide (LPS) or saline to compare efficacy of both techniques. Inflammatory cell numbers in bronchoalveolar lavage were quantified 24 h post-administration.

Evans Blue staining revealed a more homogeneous distribution and less variability among animals treated using IMIT as compared to OA. Higher inflammatory cell numbers were observed in IMIT mice compared to OA mice after exposure to vehicle or the lowest LPS concentration.

This study shows that the optimized IMIT is superior to OA with regards to efficacy, reproducibility and accuracy. This IMIT method can be deployed to refine 3R animal welfare aspects of the experimental design and improve the reproducibility of respiratory disease mouse models.

1. Introduction

In respiratory pre-clinical research, the use of mice is vital to model several pulmonary diseases. Even though there are both anatomical and immunological differences between humans and mice (Mizgerd & Skerrett, 2008), studies in mice are of great importance to the understanding of a major range of airway diseases and pathological processes that cannot be studied in humans. Still, there is a continuous need to refine current techniques and methods to achieve improved models of airway diseases to investigate disease processes in mice with reduced variability. Improved techniques will reduce the handling stress and ameliorate reproducibility of the model and consequently will contribute to the 3R principles in animal research.

One of the challenges in models for respiratory conditions is the administration of substances into the lungs in the most accurate and reproducible manner to induce a specific pulmonary pathology or to assess the efficacy of a specific treatment. A common compound applied to mimic respiratory diseases is the endotoxin lipopolysaccharide (LPS), a component of gram-negative bacteria that activates the innate immune system through binding to the toll-like receptor 4 (Poltorak et al., 1998). It is frequently used to induce acute lung injury and (chronic) lung inflammation (Rittirsch et al., 2008; Vernooy, Dentener, Van Suylen, Buurman, & Wouters, 2002) to study processes involved in for instance asthma and chronic obstructive pulmonary disease (COPD). In addition,

* Corresponding author at: Div. of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, the Netherlands.
E-mail address: c.e.pelgrim@uu.nl (C.E. Pelgrim).

https://doi.org/10.1016/j.vascn.2022.107156
Received 18 November 2021; Received in revised form 17 January 2022; Accepted 21 January 2022
Available online 25 January 2022
1056-8719/© 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

LPS is also applied as a second hit trigger to mimic COPD exacerbations (Ceelen et al., 2017; Cervilha et al., 2019; Kobayashi et al., 2013). Furthermore, LPS might be preferred instead of bacteria itself because of its safety. The proper instillation of LPS is of importance since exposure to other mucosal sites, such as the gastrointestinal tract due to overspill into the esophagus, may interfere with the model or treatment that is assessed.

A common technique to instill substances into the lungs is the oropharyngeal aspiration (OA), in which the solution is applied on the back of the tongue and inhaled by the mouse itself (Rao et al., 2003). This technique is easy to perform and is not time consuming. However, with this method it is likely that there is overspill to the gastrointestinal tract because of residues that are present in the mouth which are swallowed after the procedure. This is problematic as accurate dosing within the lungs is not secured, and the effects within the gastrointestinal tract can interfere with the disease model and/or treatment. Because of the lower accuracy achieved by the OA, the intubation-mediated intratracheal (IMIT) administration, in which the solution is administered via the trachea into the lungs, might be preferred (Lawrenz, Fodah, Gutierrez, & Warawa, 2014; Vernooy, Dentener, Van Suylen, Buurman, & Wouters, 2001). In addition, the administration of a solution into the lungs can be performed in a more standardized manner by using intubation. Comparison between OA and IMIT administration with respect to reproducibility, accuracy and efficacy is relevant to understand the advantages and disadvantages of both techniques and the optimal application under specific experimental conditional models.

With intubation, there is a risk of insertion into the esophagus, and an additional validation of proper insertion into the trachea might be desired to secure instillation into the lungs. One way to achieve this is to incorporate a mechanical breathing device after intubation in order to both validate proper insertion into the trachea and maintaining anesthesia by supplying gas anesthesia during validation.

In this study, we present an optimized protocol to administer solutions into the lungs of mice using the IMIT administration and compared the accuracy and variability of this technique with OA using Evans Blue dye and LPS administration.

2. Methods

All experimental procedures were approved by the Central Committee for Animal Experiments (CCD) and Animal Welfare Body (IVD) of the Utrecht University, the Netherlands (AVD1080020184785), and performed according to the guidelines. The study was carried out and reported in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Mice were group housed (n = 4–5 mice per cage) under standard conditions with a 12 h day-night light cycle in open cages with ad libitum access to water and food (Ssniff Spezialdiäten GmbH, Soest, Germany).

2.1. Evans blue administration

The accuracy between the techniques was compared by evaluating the distribution of the instillations throughout the lungs using Evans Blue dye. In this separate experiment, female Balb/cAnNCrl surplus mice (n = 10) between 7 and 9 months old were randomly divided to one of the two administration groups. Mice were i.t. treated with 50 μl Evans Blue solution (10 mg/ml in 0.9% NaCl; Sigma-Aldrich, the Netherlands) using either IMIT or OA. For the Evans Blue administration using the IMIT administration, a 1 ml syringe was used to administer the solution together with 250 μl air to overcome the distance in the cannula. After administration, as described in downstream sections, mice were able to recover in order to resume the normal breathing and mice were sacrificed within 30 min after administration using an overdose of pentobarbital injected intraperitoneally (i.p.) (700 mg/kg; Euthanimal, Alfasan Diergeneesmiddelen BV, Woerden, the Netherlands). Subsequently, the lungs and gastrointestinal tract were isolated in order to observe the degree of Evans Blue distribution and overspill, respectively, between and within techniques.

2.2. Lipopolysaccharide (LPS) administration and bronchoalveolar lavage (BAL)

To compare the efficacy between both techniques, the degree of inflammation after administration of saline or different concentrations of LPS in the lungs was compared. Female Balb/cByJ mice (n = 40; Charles River Laboratories, France) of 9–11 weeks old were used. Mice were randomly divided into 4 groups for each technique: treatment with vehicle or LPS (1, 10 or 100 μg/ml, corresponding to a dose of approximately 0.0023, 0.023 and 0.23 mg/kg) using either IMIT or OA. Mean body weights at baseline did not significantly differ between groups. LPS (from *Escherichia coli* 055:B5, Sigma-Aldrich, the Netherlands) was dissolved in 0.9% NaCl, and mice were either treated with 50 μl LPS solution (1, 10 or 100 μg/ml) or saline (0.9% NaCl) using IMIT or OA. Approximately 24 h after LPS administration, mice were sacrificed with an overdose of pentobarbital i.p. (700 mg/kg; Euthanimal, Alfasan Diergeneesmiddelen BV, Woerden, the Netherlands). A cannula was inserted into the trachea and lungs were lavaged four times with 1 ml fresh 0.9% NaCl (37°C) for each lavage. The average recovery of the lavage fluid was approximately 60–70%. Lavages were centrifuged (400 ×g, 5 min), pellets were resuspended in Türk solution and the total BAL cell counts were determined using the Corning Cell Counter and CytoSMART™ software (CytoSMART Technologies, Eindhoven, The Netherlands). Cytospins were prepared and stained with DiffQuick™ (Merz and Dade A.G., Düdingen, Switzerland). A blinded observer differentiated the cells into macrophages, neutrophils and lymphocytes in each sample using standard morphology. Cell numbers were counted in a total of around 200 cells and the absolute number for each cell type was calculated based on the total BAL cell counts of the corresponding sample.

2.3. Intubation-mediated intratracheal (IMIT) administration

2.3.1. Equipment list

- IV cannula, 22 gauge (G) (Abbocath™-T Venisystems, Hospira, Lake Forest, IL, USA)
- Hamilton Gastight syringe, 100 μl (Hamilton, Bonaduz, Switzerland)
- Hamilton small hub RN blunt needle, 24 G, 54 mm (Hamilton, Bonaduz, Switzerland)
- MiniVent mouse ventilator (Harvard Apparatus, Holliston, MA, USA)
- Polyethylene tubing and plastic connectors
- Induction chamber for small animals
- Veterinary anesthesia machine
- Blunt forceps
- Mouse intubation stand
- Fiber optic illuminator

2.3.2. Cannula

The cannula required some modifications prior to the procedure. First, the 24 G needle inside the cannula was made shorter and blunt to fit the size of the cannula. Secondly, the plastic cover supplied with the Abbocath cannula was cut to a size of 17 mm (based on an adult female Balb/c mouse) and applied to the cannula as an additional control to prevent the cannula from passing or getting blocked at the tracheal bifurcation.

2.3.3. The setup

Polyethylene tubing and connectors were used to make a connection between the veterinary anesthesia machine, anesthesia induction chamber, the mechanical ventilator and the cannula (Fig. 1). Both the mechanical ventilator and anesthesia induction chamber were connected to the anesthesia machine. Y-shaped tubing and a connector were

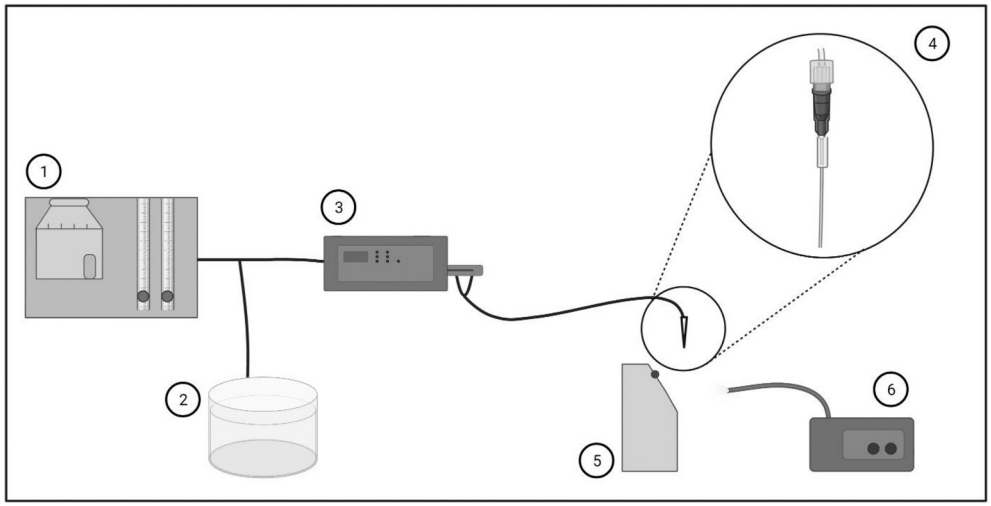

Fig. 1. Setup of the optimized IMIT administration technique. Gas anesthesia flows from the anesthesia unit (1) to both the induction chamber (2) and the mechanical breathing device (3). From the mechanical breathing device, there is a connection with the modified IV cannula (4), allowing the supplementation of isoflurane via the cannula. The mouse is positioned vertically by hanging the fore teeth of the upper jaw over a plastic wire (black dot) attached to an intubation stand with a slightly angled platform (5). A fiber optic illuminator (6), which is positioned towards the neck area of the mouse, was used to visualize the trachea. The figure was created with BioRender.com.

applied to connect the cannula to the mechanical ventilator.

2.3.4. Procedure

1. The mouse was anesthetized using isoflurane (4–5% isoflurane/air for several minutes). Once adequate anesthesia was observed, the mouse was vertically (head up) and posterior positioned using the mouse intubation stand (Fig. 1[5]).
2. A plastic wire was positioned under the fore teeth of the upper jaw by which the mouth spontaneously opened.
3. A blunt forceps was used to carefully pick up the tongue. Thereafter, the tongue was positioned at one side of the mouth by hand.
4. The fiber optic illuminator was positioned towards the neck area to illuminate the tracheal opening (Fig. 1[6]).
5. Once the tracheal opening was clearly visible, the cannula was inserted into the trachea.
6. When the cannula was maximally inserted, i.e. until the additional plastic cover reached the mouth, the needle inside the cannula was removed leaving the plastic cover inside the trachea. The tubing was then attached to the cannula, connecting the ventilator to the cannula, allowing the mechanical ventilation to be visualized. When the needle was not removed, the mechanical ventilation was not visible, possibly due to low force by high counter pressure of the needle.
7. Proper insertion of the cannula, i.e. into the trachea instead of the esophagus, was confirmed by observation of movement of the chest at the same rate as the mechanical ventilator (175 strokes/min, 200 μl). Only several seconds are required for this. Since the mechanical ventilator is attached to the anesthesia machine, gas anesthesia will be supplied during this procedure, thereby maintaining anesthesia.
8. When the cannula was successfully inserted into the trachea the connection with the ventilator was removed from the cannula and a syringe needle was inserted into the cannula after which 50 μl of the solution together with 50 μl air was injected. Finally, the syringe and cannula were removed, and the mouse was placed back in the home cage. Optionally, for example when there are signs of difficulties with anesthesia, the mouse can be placed on a heating pad until the first signs of recovery occurred. Recovery was achieved within 5 min after instillation.

2.4. Oropharyngeal aspiration (OA)

2.4.1. Equipment list

- Pipette
- Veterinary anesthesia machine
- Induction chamber for small animals
- Blunt forceps
- Mouse intubation stand

2.4.2. Procedure

1. The mouse was anesthetized using isoflurane (4–5% isoflurane/air for several minutes). Once adequate anesthesia was observed, the mouse was vertically positioned using the mouse intubation stand (Fig. 1[5]).
2. The blunt forceps was used to carefully pick up the tongue. Thereafter, the tongue was positioned at one side of the mouth by hand.
3. Using a pipette, 50 μl of the solution was pipetted at the back of the tongue.

Note: when there are problems with inhaling the solution, the nostrils of the mouse may be pinched until the mouse has inspired. This will force the mouse to breathe only through the mouth which may help instillation.

4. The tongue was maintained in this position until the mouse had inspired and thus inhaled the solution. After that, the mouse was removed from the stand and put back in the home cage. Recovery was achieved within 5 min after instillation

2.5. Statistical analyses

For comparisons between administration methods for vehicle or each concentration of LPS, independent samples *t*-tests were used. When data did not follow a normal distribution, the Mann-Whitney test was used. This applied for macrophage numbers with the 10 μg/ml LPS dose. Differences were considered significant when $p < 0.05$. For BAL cells, there was one dropout in the vehicle exposed IMIT treated group, because of an unforeseeable error occurring during BAL harvest leading to inaccurate cell numbers. Data are presented as mean ± SEM. Graphpad Prism version 9.1 was used for all statistical analyses.

3. Results

3.1. Accuracy

In order to visualize the distribution and final locations of the solutions when administered using IMIT or OA, Evans Blue solution was used. For the OA, an uneven staining was observed throughout the lungs (Fig. 2 lower left panel). Whereas administration using IMIT showed

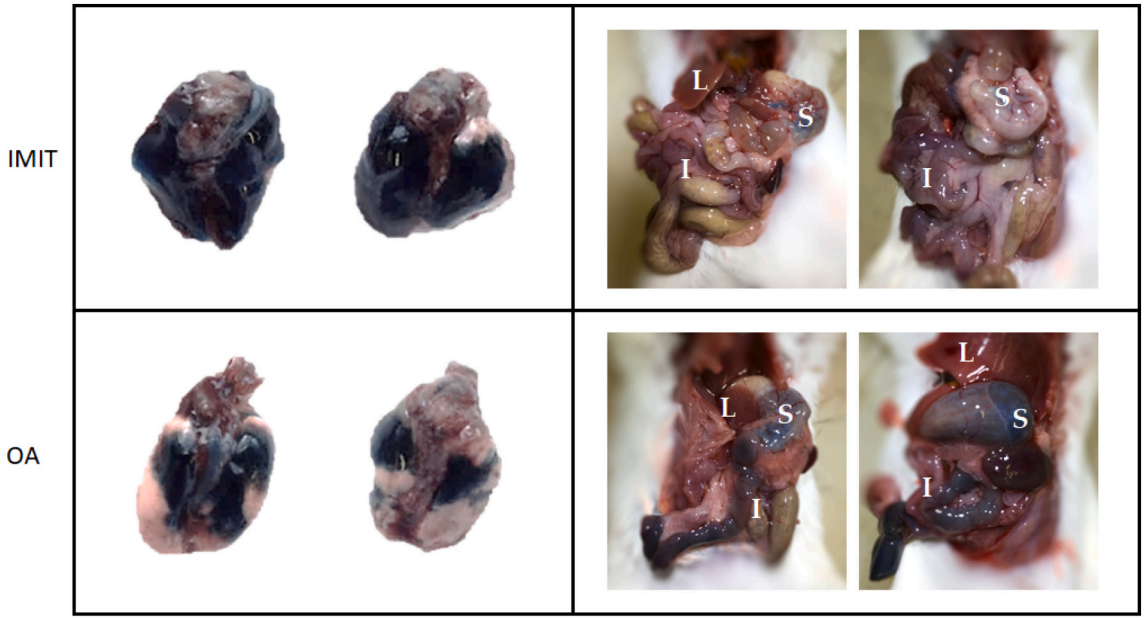

Fig. 2. Evans Blue instillation was performed in 5 animals for each procedure. Evans Blue staining shows the distribution of the administered solution between the intubation mediated intratracheal administration (IMIT; upper left panel) and oropharyngeal aspiration (OA; lower left panel). There is a difference in the distribution of Evans Blue throughout the lungs between the two procedures. Greater overspill of Evans Blue solution to the gastrointestinal tract was observed with the OA procedure (lower right panel) compared to IMIT (upper right panel). I = intestine; L = liver; S = stomach. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that Evans Blue was homogeneously distributed throughout the lungs (Fig. 2 upper left panel). In addition, higher inter-animal variation in the distribution and load of Evans Blue staining was observed in OA treated mice compared to IMIT mice. For the animals that underwent OA, stomachs were completely stained with Evans Blue in 80% of the animals, whereas in the mice treated using IMIT this complete staining of the stomachs was not observed (Fig. 2 right panels). This observation indicates that a part of Evans Blue solution was either swallowed or leaking into the esophagus after OA.

3.2. Efficacy

Administration of LPS induces a bacterial-like inflammatory response in the lungs. For this reason, LPS was used as a bacterial trigger to compare the efficacy between the IMIT and OA techniques. Approximately 24 h after administration, mice were sacrificed, and BAL was collected in order to quantify the total and specific types of inflammatory cell numbers.

Both administration routes showed a dose-dependent increase in total BAL cells (Fig. 3A). A significant higher number of total BAL cells was observed in the IMIT mice treated with either vehicle ($p < 0.05$) or 1 μg/ml LPS ($p < 0.01$) compared to mice administered using OA. For the highest concentration of LPS, 100 μg/ml, no significant differences were observed, although the variability was higher in the OA compared to the IMIT group (mean ± SEM: $515.4*10^4 \pm 57.2*10^4$ vs $471.3*10^4 \pm 18.9*10^4$ respectively). After differential count of specific cell types, both administration routes showed an LPS dose-dependent increase in BAL neutrophils in mice (Fig. 3B). The observed difference in total cell numbers between IMIT and OA groups treated with 1 μg/ml LPS was mainly because of differences in neutrophil numbers ($p < 0.01$). Again, a higher variability in neutrophil numbers was observed for the highest concentration of LPS, 100 μg/ml, in animals treated using OA compared to IMIT (mean ± SEM: $475.3*10^4 \pm 52.1*10^4$ vs $422.8*10^4 \pm 21.0*10^4$ respectively). Both administration routes showed a dose-dependent increase in BAL lymphocyte numbers, however, no differences in lymphocyte numbers were observed regarding administration routes (Fig. 3C). The difference in total BAL cell numbers for the vehicle groups was accounted for by macrophages ($p < 0.01$; Fig. 3D). No dose-dependent change in macrophages was observed in the mice treated by either administration route.

4. Discussion

In this study, an optimized technique to instill solutions in the lungs of mice, the IMIT administration, has been presented and compared to the OA procedure. The findings from this study indicate that with the IMIT instillation a relative larger part of the administered solution ends up deep in the lungs with a higher dispersion and lower variability as compared to OA. This is based on a more dense and evenly dispersed spread of Evans Blue over the lungs by the IMIT administration. In addition, more overspill to the gastrointestinal tract was observed by OA as compared to IMIT.

BAL inflammatory cell numbers after LPS administration using the IMIT procedure demonstrated differences in efficacy at a low dose: administration of 50 μl of the lowest LPS concentration, 1 μg/ml, induced a higher inflammatory cell influx in BAL as compared to OA. This difference was related to increased neutrophils numbers, indicating an LPS-specific effect, and it might be that for OA, the solution does not end up as deep into the lungs as IMIT. However, this difference was not observed for the higher doses of LPS. A reason might be that although the LPS was not completely distributed over the lungs after OA within 30 min, as indicated by Evans Blue dye, LPS may diffuse across the lungs during the following hours. In addition, a higher dose of LPS administered locally might induce an as high neutrophil influx as the same concentration which is evenly distributed over the lungs. Based on the observations, a lower neutrophil influx after OA was expected since the Evans Blue staining showed overspill to the stomach, indicating that part of the LPS might end up in the gastrointestinal tract. This is further supported by the observation that mice treated with vehicle by IMIT also displayed higher inflammatory cell numbers in the BAL as compared to mice treated through OA. This increase in inflammatory cells by saline was mainly dependent on macrophage influx, as the number of macrophages was higher in mice that underwent IMIT as opposed to OA treated mice. Saline has a relatively low pH of around 5 due to the

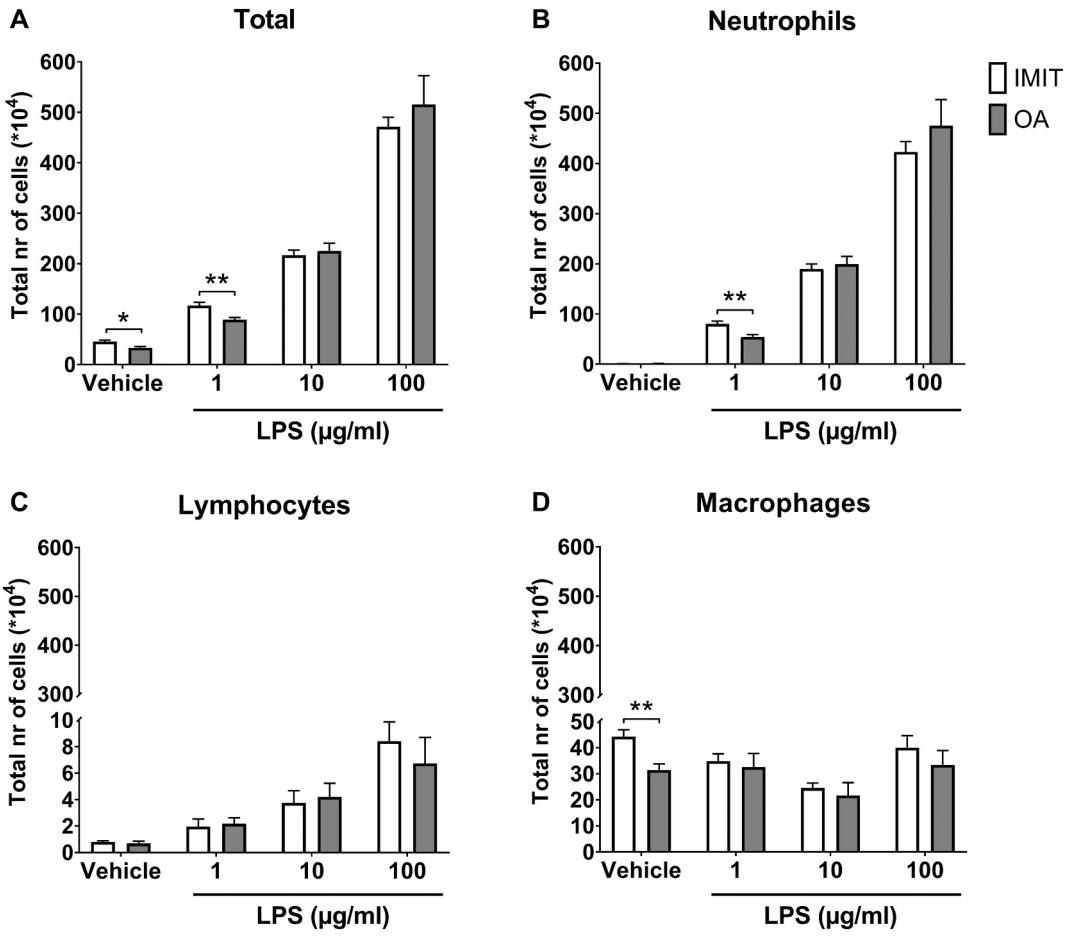

Fig. 3. Number of inflammatory cells in BAL 24 h after LPS administration. Mice were treated with 50 µl vehicle or LPS (1, 10 or 100 µg/ml) via oropharyngeal aspiration (OA; $n = 6$ per group) or intubation mediated i.t. administration (IMIT; $n = 5$ in vehicle group, n = 6 for other). After 24 h, BAL was collected, and the total number of inflammatory cells (A) was determined. In addition, neutrophils (B), lymphocytes (C) and macrophages (D) have been quantified from the total BAL cells. Data are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$.

dissolved carbon dioxide (Reddi, 2013), and the acidity of the vehicle solution might induce a mild inflammatory response through the influx of macrophages. Furthermore, LPS did not affect the number of macrophages, which has been observed after a single administration via oropharyngeal aspiration before (You, Lee, Taylor-Just, Linder, & Bonner, 2020). After a single LPS administration into the airways the majority of inflammatory cells in BAL comprises of neutrophils (Allen, 2014; Rowe, Allen, Ridger, Hellewell, & Whyte, 2002), and this was observed in the current study as well. The IMIT instillation might be favorable compared to OA because of small inter-administration and inter-experimenter differences (Driscoll et al., 2000). In this study, the lower variability was demonstrated as the standard error of the mean of the total cells in BAL for the highest LPS dose was around three times higher in OA treated animals compared to animals that underwent IMIT.

For proper interpretation of experimental data and unraveling the mechanism of a specific effect, it is essential to know precisely where a trigger or treatment effect originates from. In the recent years, focus on investigating the cross-talk between organs and tissues is increasing, in particular between the lung and gut (Enaud et al., 2020; Zhang et al., 2020). Therefore, it is crucial to accurately allocate and secure the proper dosing of the trigger and/or treatment. Oral administration of LPS both activates and suppresses specific immune responses in the gut, which can interfere with the development of disease models relating to allergy and infection (Inagawa, Kohchi, & Soma, 2011). Local LPS administration into the stomach leads to the induction of gut inflammation and has been related to changes in taste in mice, which is problematic in nutritional intervention studies (Pittman et al., 2020). In addition, gastric administration of LPS leads to changes in behavior, including increased anxiety-like behavior (Fields et al., 2018). Thus, for pulmonary research, it is desired to administer compounds into the lungs without overspill to the gastrointestinal tract. In this study, it was demonstrated that with the IMIT administration, the contamination of LPS into the gastrointestinal tract was minimal and substantially less as compared to the one observed after OA. The overspill of the instilled solution containing possible harmful compounds is crucial to consider in studies relating to the lung-gut crosstalk and specific disease models.

In this study, the OA and IMIT instillation have been compared directly. It is important to compare the accuracy and efficiency of these techniques in one study design as the literature on the separate techniques is not sufficient to make a proper comparison. Factors like different laboratory conditions can interfere with the interpretation, and a direct comparison within one laboratory will overcome interference of these factors. Side-by-side comparisons have been performed in previous studies, with conflicting conclusions (Barbayianni, Ninou, Tzouvelekis, & Aidinis, 2018; Bergamini et al., 2021; Kunda, Price, & Muttil, 2018; Lakatos et al., 2006). Some factors may have led to contradictions between studies, including type of anesthesia and the equipment used to administer the solution. Throughout the years, the IMIT procedure has been refined and optimized to benefit animal welfare and accuracy. For example, the access to the lungs in i.t. administration is performed in a non-invasive manner, i.e. without surgical opening of the trachea. In addition, gas anesthesia is frequently being applied instead of injectable anesthesia (Khadangi et al., 2021; Lawrenz et al., 2014; Vandivort, An, & Parks, 2016). Next to the shorter recovery – leading to less discomfort

and a swift recovery of the normal breathing frequency – findings from a study in mice indicate that the tidal volume is higher when using isoflurane compared to injection anesthesia using ketamine and xylazine (Tsukamoto, Serizawa, Sato, Yamazaki, & Inomata, 2014). The solution can therefore distribute throughout the lungs in a more physiological manner. Finally, in the current study, a mechanical ventilation device was added to the procedure to validate the proper insertion of the cannula in the trachea. Without this validation, it cannot be ensured the cannula is placed correctly until after the administration. A similar confirmation method using air has been described before (Qiu, Liao, Chow, & Lam, 2020), and other methods using a syringe or tubing containing a drop of (dye) solution to verify respiration have been described (Lawrenz et al., 2014; Vandivort et al., 2016). The advantage of including a mechanical breathing device is that this device is connected to the anesthesia supply, and isoflurane is simultaneously applied during the validation step to maintain anesthesia during administration, without hampering respiration. This will prevent the mouse from waking up during the administration of the solution, resulting in less discomfort to the animals. The above optimizations have not been applied simultaneously in the side-by-side comparison between the OA and IMIT before, until now. It is of importance to keep refining, optimizing and comparing protocols to select the most suitable technique for a specific experimental setup, and to secure the quality of in vivo research. In addition, in the current study, a piece has been attached to the cannula which prevents unilateral insertion of the cannula. Since proper instillation into the lungs is ensured with these modifications, little errors will occur and therefore the number of animals required will be further minimized.

Although IMIT instillation has a higher reproducibility than OA with small inter-administration and inter-experimenter differences (Driscoll et al., 2000), there are also some disadvantages of this technique. Firstly, the intubation itself is technically challenging and requires some training from the experimenter. Insufficient training can lead to increased risk of trauma caused by failed intubations (Lawrenz et al., 2014). Secondly, the IMIT administration might not be suitable for experimental setups which require daily treatment because of the possibility to develop local inflammation from the intubating cannula.

Optimization and comparison of techniques for the instillation of solutions in the airways in animal models of respiratory diseases, including acute lung injury and chronic lung diseases, is vital for reproducibility of results and the contribution to the 3R principles, including reduction and refinement. In this way, therapeutic interventions can be improved and/or developed in the most optimal manner with high relevance for future clinical studies. Depending on the experimental setup, the presented IMIT administration might be considered because of its precise and highly efficacious delivery of solutions into the lungs.

Funding

This work has been supported by the LSH-TKI Lung Foundation PPP allowance 10.2.16.119.

Declaration of competing interest

J.G. is part-time employee of Danone Nutricia Research, Utrecht, the Netherlands. A.H. is employee of Danone Nutricia Research, Utrecht, the Netherlands. The other authors declare no competing interests.

References

Allen, I. C. (2014). The utilization of oropharyngeal intratracheal PAMP administration and bronchoalveolar lavage to evaluate the host immune response in mice. *JoVE*, (86).

Barbayianni, I., Ninou, I., Tzouvelekis, A., & Aidinis, V. (2018). Bleomycin revisited: A direct comparison of the intratracheal micro-spraying and the oropharyngeal aspiration routes of bleomycin administration in mice. *Frontiers in Medicine, 5*, 269.

Bergamini, G., Perico, M. E., Di Palma, S., Sabatini, D., Andreetta, F., Defazio, R., … Ferrari, L. (2021). Mouse pneumonia model by Acinetobacter baumannii multidrug resistant strains: Comparison between intranasal inoculation, intratracheal instillation and oropharyngeal aspiration techniques. *PLoS One, 16*(12), Article e0260627.

Ceelen, J. J., Schols, A. M., Van Hoof, S. J., de Theije, C. C., Verhaegen, F., & Langen, R. C. (2017). Differential regulation of muscle protein turnover in response to emphysema and acute pulmonary inflammation. *Respiratory Research, 18*(1), 1–10.

Cervilha, D. A., Ito, J. T., Lourenço, J. D., Olivo, C. R., Saraiva-Romanholo, B. M., Volpini, R. A., … Tibério, I. F. (2019). The Th17/Treg Cytokine Imbalance in Chronic obstructive pulmonary disease exacerbation in an animal model of cigarette smoke exposure and lipopolysaccharide challenge association. *Scientific Reports, 9*(1), 1–13.

Driscoll, K. E., Costa, D. L., Hatch, G., Henderson, R., Oberdorster, G., Salem, H., & Schlesinger, R. B. (2000). Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: Uses and limitations. *Toxicological Sciences, 55*(1), 24–35.

Enaud, R., Prevel, R., Ciarlo, E., Beaufils, F., Wieërs, G., Guery, B., & Delhaes, L. (2020). The gut-lung axis in health and respiratory diseases: A place for inter-organ and inter-kingdom crosstalks. *Frontiers in Cellular and Infection Microbiology, 10*, 9.

Fields, C. T., Chassaing, B., Castillo-Ruiz, A., Osan, R., Gewirtz, A. T., & de Vries, G. J. (2018). Effects of gut-derived endotoxin on anxiety-like and repetitive behaviors in male and female mice. *Biology of Sex Differences, 9*(1), 1–14.

Inagawa, H., Kohchi, C., & Soma, G.-I. (2011). Oral administration of lipopolysaccharides for the prevention of various diseases: Benefit and usefulness. *Anticancer Research, 31*(7), 2431–2436.

Khadangi, F., Forgues, A.-S., Tremblay-Pitre, S., Dufour-Mailhot, A., Henry, C., Boucher, M., … Brunet, D. (2021). Intranasal versus intratracheal exposure to lipopolysaccharides in a murine model of acute respiratory distress syndrome. *Scientific Reports, 11*(1), 1–12.

Kobayashi, S., Fujinawa, R., Ota, F., Kobayashi, S., Angata, T., Ueno, M., … Ishii, T. (2013). A single dose of lipopolysaccharide into mice with emphysema mimics human chronic obstructive pulmonary disease exacerbation as assessed by micro-computed tomography. *American Journal of Respiratory Cell and Molecular Biology, 49*(6), 971–977.

Kunda, N. K., Price, D. N., & Muttil, P. (2018). Respiratory tract deposition and distribution pattern of microparticles in mice using different pulmonary delivery techniques. *Vaccines, 6*(3), 41.

Lakatos, H. F., Burgess, H. A., Thatcher, T. H., Redonnet, M. R., Hernady, E., Williams, J. P., & Sime, P. J. (2006). Oropharyngeal aspiration of a silica suspension produces a superior model of silicosis in the mouse when compared to intratracheal instillation. *Experimental Lung Research, 32*(5), 181–199.

Lawrenz, M. B., Fodah, R. A., Gutierrez, M. G., & Warawa, J. (2014). Intubation-mediated intratracheal (IMIT) instillation: A noninvasive, lung-specific delivery system. *JoVE*, (93), Article e52261.

Mizgerd, J. P., & Skerrett, S. J. (2008). Animal models of human pneumonia. *American Journal of Physiology-Lung Cellular and Molecular Physiology, 294*(3), L387–L398.

Pittman, D. W., Dong, G., Brantly, A. M., He, L., Nelson, T. S., Kogan, S., … McCluskey, L. P. (2020). Behavioral and neurophysiological taste responses to sweet and salt are diminished in a model of subclinical intestinal inflammation. *Scientific Reports, 10*(1), 1–13.

Poltorak, A., He, X., Smirnova, I., Liu, M.-Y., Van Huffel, C., Du, X., … Galanos, C. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. *Science, 282*(5396), 2085–2088.

Qiu, Y., Liao, Q., Chow, M. Y., & Lam, J. K. (2020). Intratracheal administration of dry powder formulation in mice. *JoVE*, (161), Article e61469.

Rao, G., Tinkle, S., Weissman, D., Antonini, J., Kashon, M., Salmen, R., … Hoover, M. (2003). Efficacy of a technique for exposing the mouse lung to particles aspirated from the pharynx. *Journal of toxicology and environmental health Part A, 66*(15–16), 1441–1452.

Reddi, B. A. (2013). Why is saline so acidic (and does it really matter?). *International Journal of Medical Sciences, 10*(6), 747.

Rittirsch, D., Flierl, M. A., Day, D. E., Nadeau, B. A., McGuire, S. R., Hoesel, L. M., … Leng, L. (2008). Acute lung injury induced by lipopolysaccharide is independent of complement activation. *The Journal of Immunology, 180*(11), 7664–7672.

Rowe, S. J., Allen, L., Ridger, V. C., Hellewell, P. G., & Whyte, M. K. (2002). Caspase-1-deficient mice have delayed neutrophil apoptosis and a prolonged inflammatory response to lipopolysaccharide-induced acute lung injury. *The Journal of Immunology, 169*(11), 6401–6407.

Tsukamoto, A., Serizawa, K., Sato, R., Yamazaki, J., & Inomata, T. (2014). Vital signs monitoring during injectable and inhalant anesthesia in mice. *Experimental Animals*, 14–0050.

Vandivort, T. C., An, D., & Parks, W. C. (2016). An improved method for rapid intubation of the trachea in mice. *JoVE*, (108), Article e53771.

Vernooy, J. H., Dentener, M. A., Van Suylen, R. J., Buurman, W. A., & Wouters, E. F. (2001). Intratracheal instillation of lipopolysaccharide in mice induces apoptosis in bronchial epithelial cells: No role for tumor necrosis factor-α and infiltrating neutrophils. *American Journal of Respiratory Cell and Molecular Biology, 24*(5), 569–576.

Vernooy, J. H., Dentener, M. A., Van Suylen, R. J., Buurman, W. A., & Wouters, E. F. (2002). Long-term intratracheal lipopolysaccharide exposure in mice results in

chronic lung inflammation and persistent pathology. *American Journal of Respiratory Cell and Molecular Biology, 26*(1), 152–159.

You, D. J., Lee, H. Y., Taylor-Just, A. J., Linder, K. E., & Bonner, J. C. (2020). Sex differences in the acute and subchronic lung inflammatory responses of mice to nickel nanoparticles. *Nanotoxicology, 14*(8), 1058–1081.

Zhang, D., Li, S., Wang, N., Tan, H.-Y., Zhang, Z., & Feng, Y. (2020). The cross-talk between gut microbiota and lungs in common lung diseases. *Frontiers in Microbiology, 11*, 301.