Optimization of Tracheal Intubation and Ventilatory Management in a Rat Thoracotomy Model

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Objective: To establish a practical and reproducible method for procedural standardization during tracheal intubation and ventilatory management during thoracotomy in rats, and evaluate the invasiveness of thoracotomy as a control condition.
Methods: Tracheal length and diameter were measured in adult female Wistar rats to determine appropriate intubation devices. Positive end-expiratory pressure (PEEP) was titrated (0–5 cmH_2O) under open chest conditions using a macroscopic lung expansion score. In a separate experiment, the rats underwent left thoracotomy with incision lengths of 1, 2, or 3 cm, followed by macroscopic and histological assessments 4 weeks later.
Results: Mean tracheal length and inner diameter were 59.7 and 1.63 mm, respectively. A 16-gauge, 48-mm intravenous catheter was suitable for intubation. Optimal lung expansion was achieved at a PEEP of 4 cmH_2O. Macroscopic pleural adhesions and post-operative weight loss were not observed in any thoracotomy groups. Histological examination revealed mild pleural thickening and fibrin deposition without differences in incision length.
Conclusions: A standardized intubation and ventilatory protocol with PEEP set at 4 cmH_2O provides stable lung expansion during thoracotomy in rats. A thoracotomy up to 3 cm in length induces minimal pathological changes and is suitable as a control condition in experimental thoracic surgery models.

Key words: Rat model, Thoracotomy, Tracheal intubation, Mechanical ventilation, PEEP

INTRODUCTION

Rat models are widely used in experimental thoracic and respiratory research because the fundamental physiological mechanisms of ventilation, gas exchange, and pulmonary circulation are conserved across mammals [1, 2]. In thoracic surgical research, rat thoracotomy models are frequently employed to evaluate surgical techniques, devices, and therapeutic materials [3–5].

Despite their widespread use, there are no standardized guidelines regarding tracheal intubation devices, ventilatory settings, or degree of thoracotomy invasiveness [6]. Variability in these factors may reduce the reproducibility and complicate the interpretation of experimental outcomes. Therefore, it is essential to establish practical and reproducible procedures for airway management and ventilation of rat models.

Hence, this study aimed to optimize tracheal intubation and ventilatory management during rat thoracotomy based on quantitative anatomical measurements, and to evaluate the degree of thoracotomy invasiveness, focusing on pleural adhesions and histological changes, to determine its suitability as a control condition.

MATERIALS AND METHODS

Animals

Female Wistar rats aged 16 weeks (body weight, 180–192 g) were used in this study. Animals were housed under controlled conditions (temperature 20–26°C, humidity 35–70%, 12-hour light–dark cycle) with free access to food and water.

All experimental procedures were approved by the institutional animal care and use committee and were conducted in accordance with institutional guidelines (approval number: 211072; approval period: October 8, 2021, to March 31, 2022).

Anesthesia, tracheal Intubation, and ventilation

Anesthesia was induced with 5% sevoflurane via inhalation and maintained with 2.5–4% sevoflurane. The rats were placed on an intubation platform inclined at 45°, and the upper incisors were secured to facilitate laryngoscopic visualization. (Fig. 1A) Tracheal intubation was performed using a 16-gauge intravenous catheter sheath (outer diameter 1.7 mm, length 48 mm; Fig.

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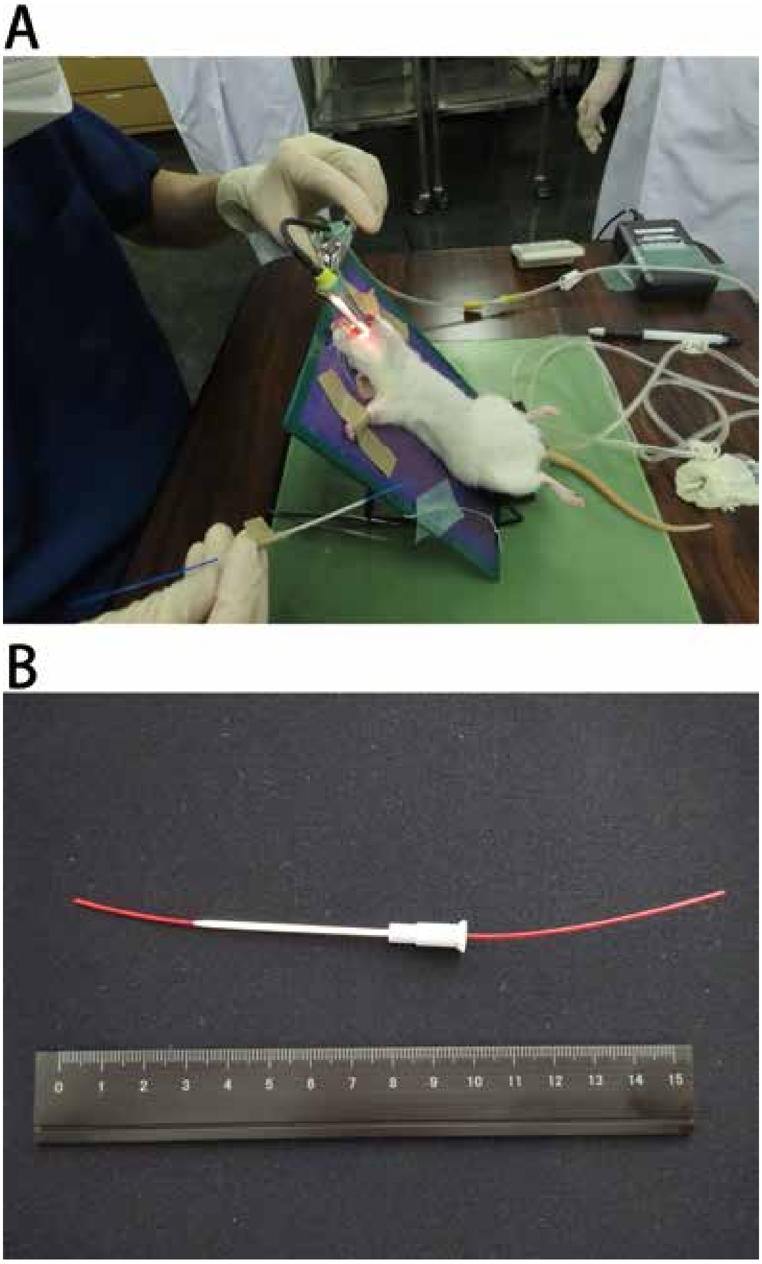

Fig. 1 Tracheal intubation setup and catheter selection in adult rats (A) Intubation was performed with the rat on a 45-degree inclined platform, with the upper incisors secured to facilitate laryngeal exposure. (B) A 16-gauge, 48-mm intravenous catheter used as an endotracheal tube, selected based on quantitative tracheal measurements.

1B). Mechanical ventilation was set at a tidal volume of 0.01 mL/g and a respiratory rate of 60 breaths/min.

Evaluation of lung expansion and positive end-expiratory pressure

Under open-chest conditions, positive end-expiratory pressure (PEEP) was increased stepwise from 0 to 5 cmH_2O in 1-cmH_2O increments. After 30 seconds of ventilation at each level, lung expansion was visually evaluated using a five-point scale (1 = complete collapse; 5 = adequate expansion). After the evaluation, the chest was closed, and the rats were allowed to recover.

This pilot evaluation was performed in a single rat as a feasibility assessment to determine a practical ventilatory setting under open-chest conditions while minimizing animal use; it was not designed for statistical comparison.

Measurement of tracheal length and diameter

After completion of the ventilation experiments, the rats were euthanized with an overdose of anesthetic agents. The distance from the incisors to the tracheal bifurcation was measured and the internal tracheal diameter was recorded.

Thoracotomy and histological evaluation

Groups A, B, and C of rats underwent left thoracotomy with incision lengths of 3, 2, and 1 cm, respectively. Macroscopic evaluation of pleural adhesions was performed. Lung tissues from the thoracotomy site were fixed with formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological assessment. Histological changes (pleural

Table 1 Distance from the upper incisors to the tracheal bifurcation and tracheal internal diameter (n = 3)

	Rat①	Rat②	Rat③	average (mm)
Distance from the upper incisors to the tracheal bifurcation (mm)	59.5	59.5	60.1	59.7 ± 0.35
Tracheal internal diameter (mm)	1.70	1.60	1.60	1.63 ± 0.06

Table 2 Effects of PEEP on lung expansion in an open-chest rat (pilot evaluation, n = 1)

PEEP (cmH_2O)	0	1	2	3	4	5
lung expansion (1–5)	3	3	3	4	5	5

1 = complete collapse 5 = adequate expansion

Fig. 2 Pilot evaluation of PEEP during open-chest ventilation
Macroscopic assessment of lung expansion under left thoracotomy conditions at different PEEP levels. Adequate bilateral lung expansion was achieved at a PEEP of 4 cmH_2O in this pilot evaluation. PEEP, positive end-expiratory pressure.

thickening, fibrin deposition, and inflammatory cell infiltration) were evaluated qualitatively; formal quantitative scoring was not performed because these findings were focal and varied depending on the sectioning plane.

Statistical Analysis

Group comparisons were performed using unpaired t-tests with the Bonferroni correction for multiple comparisons. Statistical significance was set at $p < 0.05$.

RESULTS

Tracheal measurements and selection of the intubation device

The mean distance from the incisors to the tracheal bifurcation was 59.7 mm (range 59.5–60.1 mm), and the mean tracheal internal diameter was 1.63 mm (range 1.6–1.7 mm) (Table 1). Based on these measurements, a 16-gauge, 48-mm catheter sheath was considered appropriate for tracheal intubation of adult female Wistar rats (Fig. 1).

Pilot assessment of PEEP for lung expansion

In the pilot evaluation, adequate lung expansion (score 5) was observed at PEEP levels of 4 and 5 cmH_2O (Table 2). To minimize the potential risk of overdistension, we selected a PEEP of 4 cmH_2O as a practical working setting under open-chest conditions (Fig. 2).

This pilot observation was used to select a working PEEP. A PEEP of 4 cmH_2O was subsequently applied in the thoracotomy experiments in this study and provided stable lung expansion without apparent ventilation-related complications.

Invasiveness of Thoracotomy

One rat subjected to the 1-cm thoracotomy (group C) died because of anesthetic complications; all remaining animals survived until evaluation. No macroscopic pleural adhesions were observed in any group after four weeks. Histological examination revealed mild pleural thickening, fibrin deposition, and minimal inflammatory cell infiltration at the thoracotomy sites (Fig. 3B), whereas the non-thoracotomized lung showed a normal pleural architecture (Fig. 3A). Across incision lengths (1–3 cm), these changes were mild and no apparent qualitative differences were observed in the sections examined. Likewise, no postoperative weight loss was observed in any animal (Fig. 4).

DISCUSSION

In this study, we established a practical and reproducible method for tracheal intubation and ventilatory management during thoracotomy in rats, based on

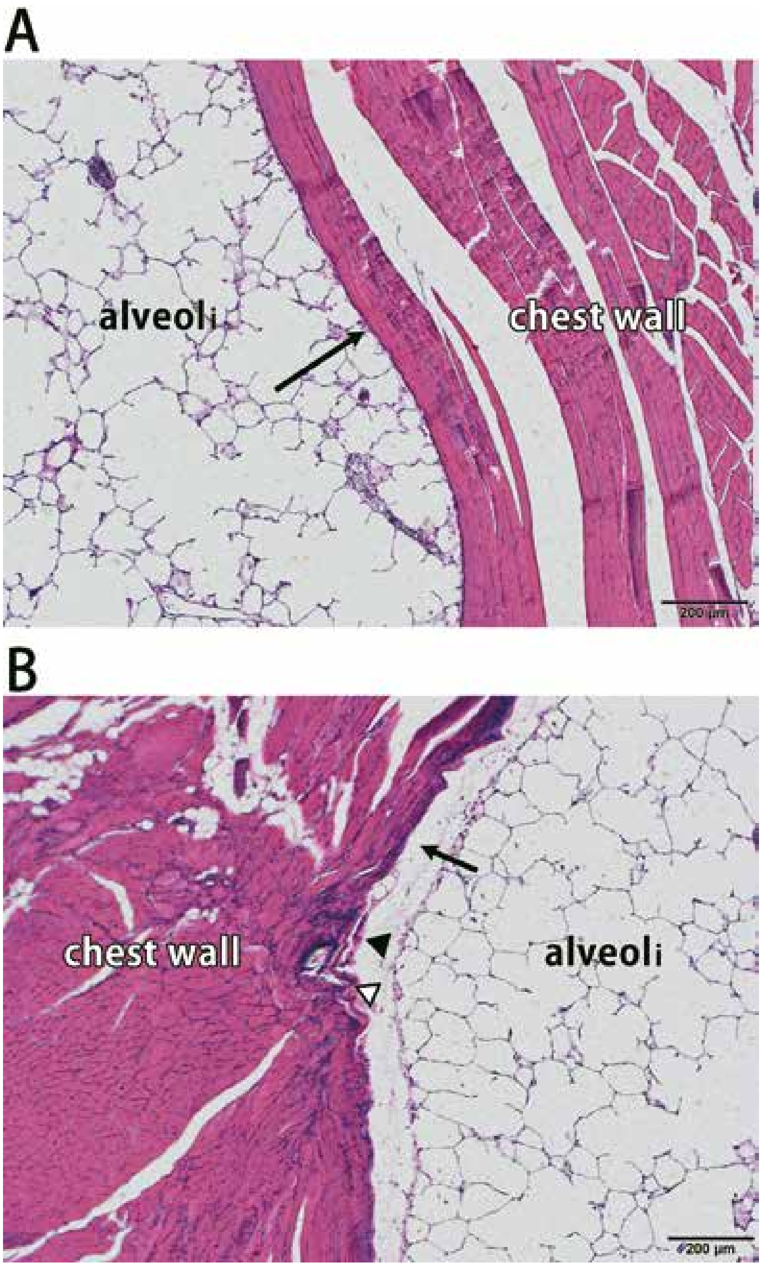

Fig. 3 Histological findings after thoracotomy
Representative hematoxylin–eosin–stained sections of the lungs and pleura.
(A)Non-thoracotomized lung showing a normal pleural architecture (↑).
(B)Thoracotomized lung demonstrating mild pleural thickening (↑) and fibrin deposition (△) with minimal inflammatory cell infiltration (▲). Similar qualitative findings were observed across incision lengths (1–3 cm). Fibroblast proliferation was not observed in any case.

direct anatomical measurements. Although the number of animals used for anatomical measurements and ventilatory assessments was limited, this study was focused on methodological standardization rather than hypothesis-driven statistical comparison. It was found that using a 16-gauge, 48-mm catheter sheath allowed for stable airway control without excessive airway trauma.

In a pilot open-chest assessment, a PEEP of 4 cmH_2O provided adequate lung expansion while avoiding the potential risk of gross overinflation at 5 cmH_2O. Lung expansion was assessed visually using a predefined scale, which is a pragmatic approach when the primary aim is to establish a practical ventilatory setting. The selected PEEP level was subsequently used in the thoracotomy experiments in this study and allowed stable procedures.

Because PEEP titration was evaluated in a single animal, we have revised the manuscript to present this as a preliminary observation and to avoid definitive wording such as "best" or "optimal." Validation in larger cohorts and quantitative functional assessments will be important in future studies.

Furthermore, our results indicate that thoracotomy itself, with incision lengths of up to 3 cm, induces only minimal macroscopic and histological changes and

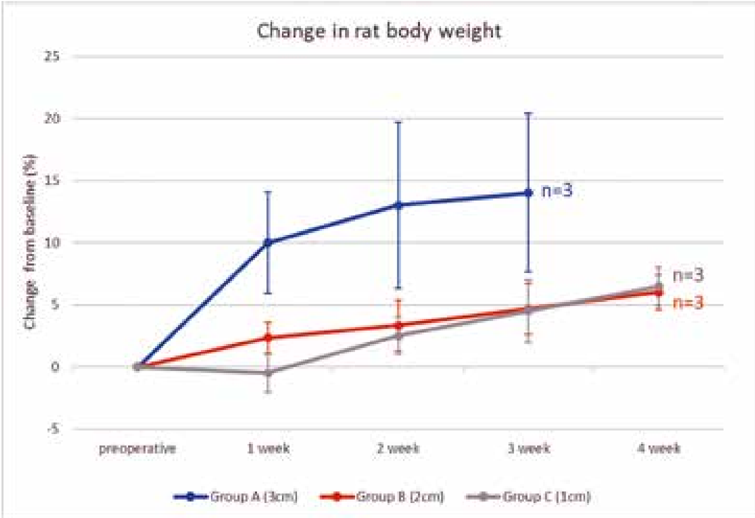

Fig. 4 Change in rat body weighta

does not result in pleural adhesion, consistent with previous observations in rat pleural injury models [6]. Although the sample size for histological evaluation was limited and formal quantitative scoring was not performed, the absence of apparent qualitative differences among the groups suggests that an incision length within this range is unlikely to be a major determinant of the postoperative pleural response. These findings support the use of the thoracotomy model developed in this study as an appropriate control for experimental thoracic studies.

In conclusion, we propose a standardized strategy for tracheal intubation and ventilatory management in rat thoracotomy based on a quantitative anatomical assessment. In our pilot evaluation, a PEEP of 4 cmH_2O served as a practical working setting for maintaining lung expansion under open-chest conditions, and a thoracotomy up to 3 cm in length can be performed with minimal invasiveness. This model is suitable as a control for future experimental studies in thoracic surgery and respiratory research; using larger cohorts and quantitative functional assessments may further refine these findings. This study provides a practical baseline framework for experimental design in rats.

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CONFLICT OF INTEREST

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