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Modification of Oropharyngeal Aspiration Technique for Mouse Using Syringe Pump

Jin-Sung Kim[1], Mi-Jin Yang[1], Sung Gu Han[2], Choong-Yong Kim[3], Sang-Sup Han[3] and Chang-Woo Song[1]

[1]Inhalation Toxicology Team, Korea Institute of Toxicology, Daejeon 305-343, Korea
[2]Graduate Center for Toxicology, College of Medicine, University of Kentucky, Lexington, KY 40536, USA
[3]Division of Nonclinical Stuides, Korea Institute of Toxicology, Daejeon 305-343, Korea

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Respirable particles cause many occupational and environmental diseases of the lung. To study these diseases, laboratory animals are often exposed to these particles. Inhalation and instillation are the well-known techniques for experimental exposures of the lung to respirable particles. Recently, another technique called oropharyngeal aspiration (OPA) has been introduced for exposing the lung to pathogens and/or particles. The conventional OPA technique for the mouse is generally carried out using a micropipette with a fixed slant board. In order to modify the conventional OPA in this study, anesthetized mice were placed on an adjustable slant board, a syringe pump was used to deliver the solution to the oropharynx, and the mice were allowed to recover in vertically positioned tubes for 6 minutes until fully awaked. Most importantly, the whole process of OPA could be carried out simply by an examiner. This modified OPA technique was validated by exposing the mouse lung to Evans Blue dye with a success rate of 95%.

Key words: Inhalation, Mouse, Oropharynx, Aspiration.

INTRODUCTION

The techniques of inhalation and intratracheal instillation (ITI) for exposing animal lungs to respirable particles have been used in order to investigate pulmonary toxicity (Hanson *et al.*, 1985; Henderson *et al.*, 1995). Inhalation studies have a high cost technical difficulty, including specialized equipments such as generators, monitoring instruments, exposure chambers and exhausts (Oberdörster, 1996). In addition, the rodent nose is much more effective than the human nose in screening out particles and, as a result, many respirable particles are removed in inhalation studies using rodents (Kimbell, 2006). Because ITI is a method to administer a respirable agent into rodent's trachea through surgical tracheostomy or intubation, it requires a high degree of technical skill with a long training period (Driscoll *et al.*, 2000). In mice, the ITI technique causes significant drawbacks such as uneven pulmonary distribution and trauma.

In humans, OPA has been identified as a natural process causing the lung to be exposed to foreign materials most frequently oropharyngeal secretions (Brain and Valberg, 1979). Experimentally controlling this process permits reproducible respirable particle exposure which bypasses the nose. OPA is a simple technique for delivering liquids or suspensions to the mouse lung and is less stressful to animals (Keane-Meyers *et al.*, 1998; Foster *et al.*, 2001; Rao *et al.*, 2003). Also, the results of OPA are better than those of ITI with more uniform distribution of particles in the lung (Shvedova *et al.*, 2005; Lakatos *et al.*, 2006).

The conventional OPA procedure may be performed by one or two experimenters. The anesthetized mouse is placed on a slant board, the tongue is gently extended by one researcher, and the solution of a test material to be aspirated is placed on the back of the tongue with a micropipette; therefore, the conventional OPA are facilitated when the pipetting is performed by the other researcher. In this study, we designed to modify the conventional method. The OPA was carried out

Correspondence to: Jin-Sung Kim, Inhalaltion Toxicology Team, Korea Insitute of Toxicology, 100 Jang-Dong, Yuseong-Gu, Daejeon, 305-343 Korea
E-mail: jskim@kitox.re.kr

Abbreviation: ITI, intratracheal instillation; OPA, oropharyngeal aspiration; EB, Evans Blue dye; PBS, phosphate-buffered saline.

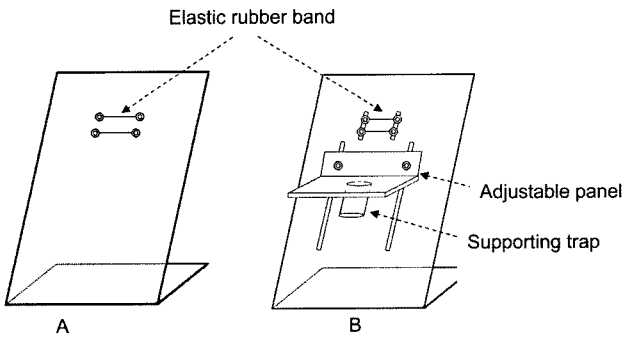


Fig. 1. Schematic illustrations of conventional slant board (A) and adjustable slant board (B).

by one experimenter using a syringe pump to administer the Evans Blue dye solution into the mouse oropharynx. The mouse was placed on an adjustable slant board with a supporting trap for inserting a holding tube. This semi-automated modification of OPA technique permitted the rapid, humane and reproducible exposure of large numbers of mice.

MATERIALS AND METHOD

Materials. Evans Blue (EB) dye and phosphate-buffered saline (PBS, pH 7.2) were supplied from Aldrich-Sigma (St. Louis, MO, USA). The syringe pump (RSC-22, Harvard Apparatus, Holliston, MA, USA) was equipped with a syringe (1001, Hamilton, Reno, NV, USA) which was connected to PEEK tubing (1531, Upchurch Scientific, Oak Harbor, WA, USA). The conventional slant board of simple structure (Fig. 1A) was changed to the adjustable slant board with a supporting trap into which the plastic tube (Falcon 50 ml, ThermoFisher Scientific, Pittsburgh, PA, USA) was inserted for holding mouse (Fig. 1B).

Animals. Specific-pathogen-free female and male ICR mice were supplied by Orient Bio (Sungnam, Korea). The animals were fed on laboratory rodent chow and watered *ad libitum*. The mice were maintained on a 12-hour light/dark cycle. All procedures using animals were reviewed and approved by the Institutional Animal Care and Use Committee and the animal program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Oropharyngeal aspiration of Evans Blue dye. The EB solutions, which were dissolved at the concentration of 0.5%, 0.25% and 0.05% in PBS, were subjected to ultrasonic agitation for 12 hours followed by tests for the distribution of the dye within the lung. Male and female mice were anesthetized with isoflurane for about 20 seconds in a glass jar with a wire-mesh floor on the bottom. The anesthetized mice were taken out of the jar and were positioned in the holding tube (Fig. 2A) which was inserted into the supporting trap attached on the adjustable slant board (Fig. 2B) The cranial incisors of anesthetized mouse were suspended by rubber band fixed on the board (Fig. 2C). The tongue was gently pulled aside from the oral cavity and extended fully using blunt small forceps in order to visualize the base of the tongue and the oropharynx (Fig. 3). A 40 μl solution of EB was delivered through PEEK tubing (ID 250 μm and OD 1.57 mm) from a syringe pump for 3 seconds. While the 40 μl solution was dropping into the posterior oropharynx, the tongue was restrained until at least two deep breaths were completed. After the EB solution was aspirated from oropharynx to larynx, the

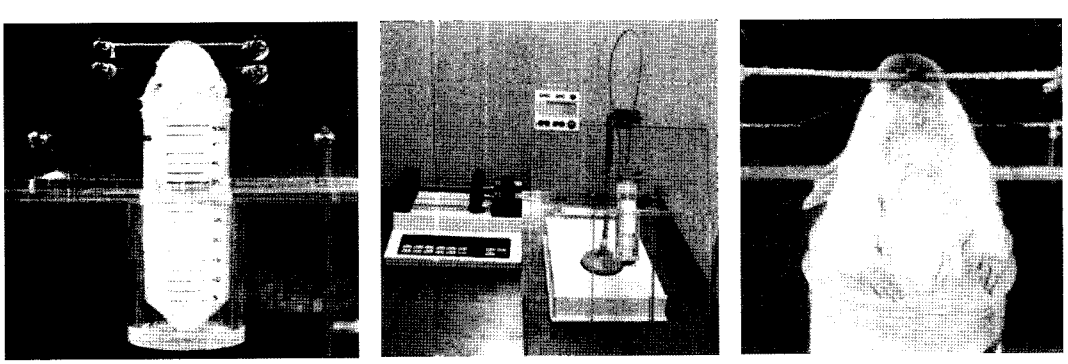


Fig. 2. (A) The mouse was placed in a holding tube which was inserted into the supporting trap, (B) the modified slant board with a holding tube and the supporting trap in the adjustable panel, and (C) cranial incisors of anesthetized mouse were suspended on a rubber band.

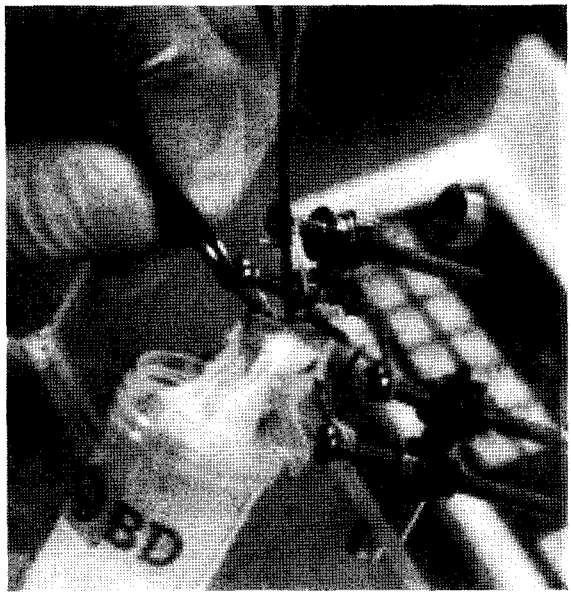


Fig. 3. The mouse tongue was gently extracted and pulled aside to visualize the base of the tongue and the oropharynx.

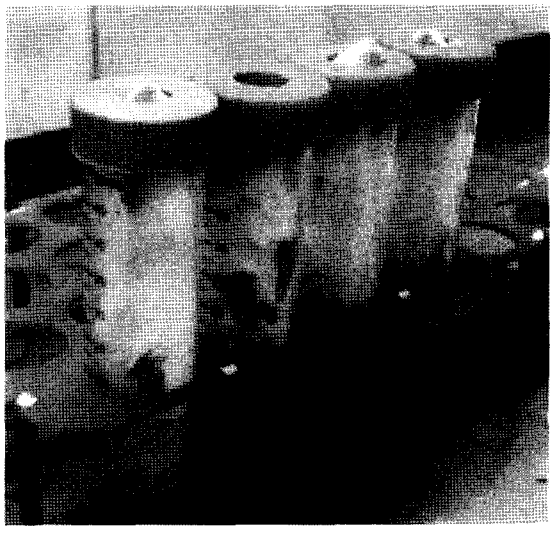


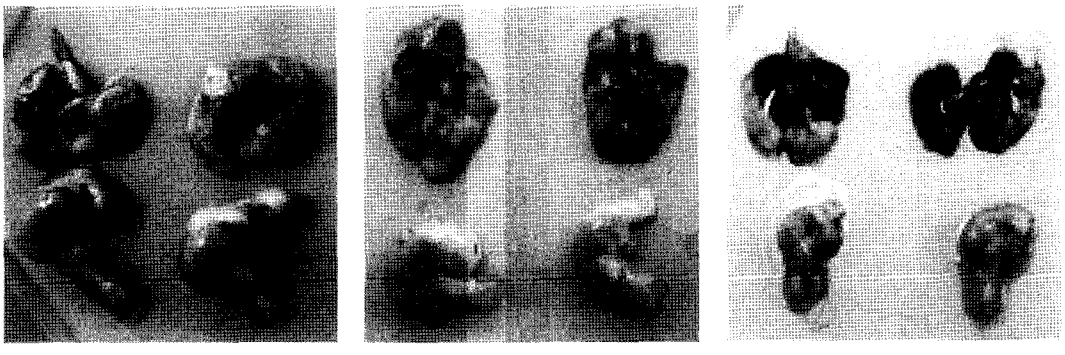
Fig. 4. The EB treated mice were placed vertically inside the holding tubes for recovery during 6 minutes.

tongue was released gently followed by positioning vertically inside a conical tube for recovery during 6 minutes (Fig. 4). The organs including lung, stomach and intestine were investigated to confirm the final location of aspirated EB according to the time intervals at 0 min, 6 min, 3 h and 24 h after exposing EB.

RESULTS

This modification of oropharyngeal aspiration was undertaken using a syringe pump to deliver the solution of EB in PBS to mouse lungs. First of all, the simple slant board structure was changed to the adjustable slant board which has an attached supporting trap. This supporting trap was adjusted along with the channels in order to make an appropriate position of the holding tube. The syringe pump was controlled to run for 3 seconds in order to deliver the EB solution into the oropharynx of the mouse placed on the adjustable slant board. The mice that aspirated the EB solution were kept in the holding tube for 6 minutes on a vertical position, and the cap of the tube were punched to make a hole for fresh air supply during the recovery time.

In the test to determine the optimal EB concentration for success rate and distribution in the lungs, EB solution of 0.05% was not enough to confirm the distribution of EB in the lungs (Fig. 5A); however this solution is better to test the success rate of aspiration using pump because there might be the least clearance effect from low concentration of EB after aspiration. EB solu-



A B C

Fig. 5. The lungs and stomachs were removed from mice that were treated with EB solution at the concentrations of 0.05 (A), 0.25 (B) and 0. 5% (C).

Table 1. The locations of EB on the organ of lung, stomach and intestine for four groups

Organ \ Group	Group I (20 mice)	Group II (6 mice)	Group III (6 mice)	Group IV (6 mice)
Lung	19	6	6	6
Stomach	1	6	6	
Intestine			6	6

*The numbers in group II, III and IV means that EB was found simultaneously in each organs.

tion of 0.25% showed an even distribution of EB with an optimal density (Fig. 5B) while EB solution of 0.5% was too high because the EB solution was found in the stomach as well as in the lung through the clearance process from mouth or trachea to stomach (Fig. 5C). Therefore, the optimal concentration of EB for testing lung distribution was concluded as 0.25%

In order to investigate the final location and distribution of administered EB using the syringe pump, lung, stomach and intestine were examined in the four groups of mice with varying recovery periods. These groups were defined as group I (0 min, 20 mice) with 0.05% EB, group II (6 min, 6 mice) with 0.25% EB, group III (3 h, 6 mice) with 0.25% EB and group IV (24 h, 6 mice) with 0.25% EB. Table 1 showed the location of EB on the organs in each group. In group I, EB was localized well only in the lungs of the 19 mice (Fig. 6A), but EB was found only in the stomach of a mouse as failure. As resulting, the success rate of modified OPA was 95% in the group I following OPA administration. However, in group II, EB was found in both lung and stomach (Fig. 6B). The trace of EB in the group III was found simultaneously in stomach and small intestine as well as in lungs (Fig. 6C). In many cases of the group IV, although the treated EB was widely distributed throughout the lung, the residual EB was not found in stomach but most of residual EB remained in the intestine (Fig. 6D).

DISCUSSION

The typical slant board with the slope angle of ~60° was frequently used in the previous studies as a conventional tool for instillation and aspiration. In this study, the simple slant board was modified using an adjustable panel with an attached supporting trap into which a holding tube was inserted. Prior to exposing the mice to the EB solution, the position of the supporting trap was adjusted for positioning the front legs of mouse on the top edge of the holding tube (Fig. 2A). Also, the diameter of the supporting trap was chosen according to the

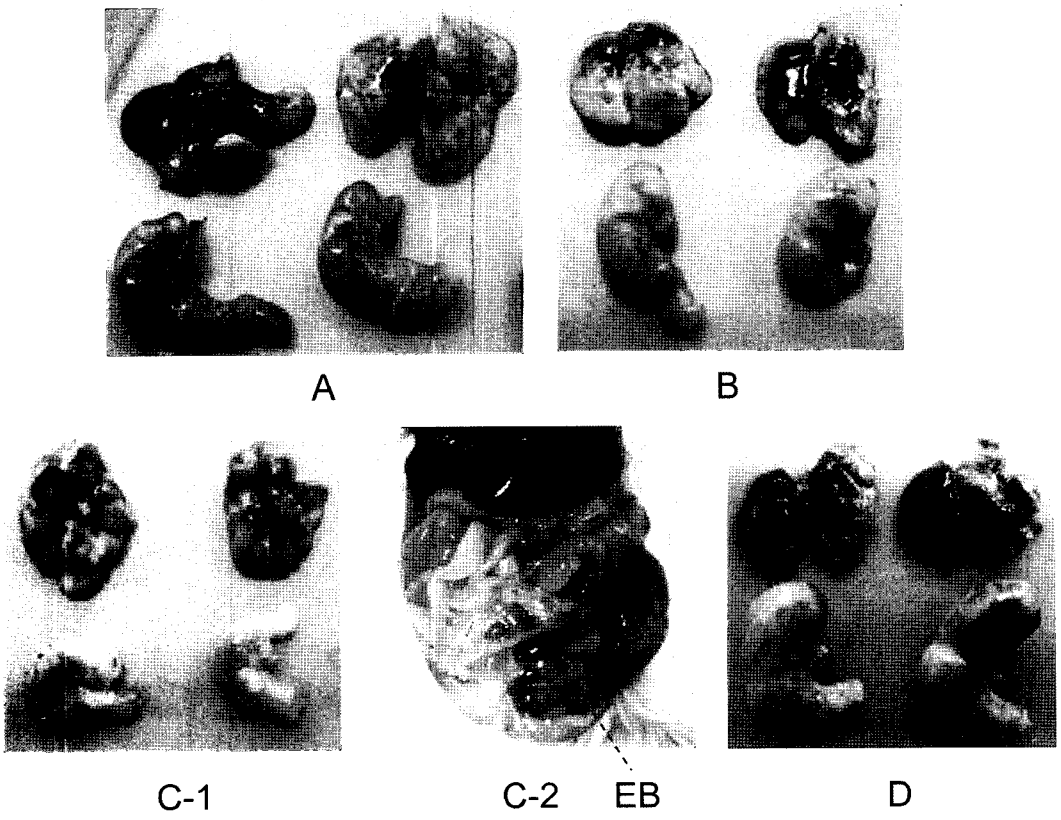


Fig. 6. The EB localization in the course of recovery and clearance; (A) 0 min, (B) 6 min, (C) 3 h and (D) 24 h.

size of animals in the holding tube. In this study, 7~8 weeks old ICR mice were used and two different sizes of holding tubes were used according to the body weights of both female and male (26~38 g and 33~45 g, respectively).

The syringe pump for the administration of 40 μl EB to lungs was controlled for 3 seconds, and the syringe was connected with PEEK tubing for EB delivery. When the EB solution was dropped into the oropharynx of mouse at the end of the tubing, a mouse breathed occasionally through the nose. In this case, the nostrils of mouse were pinched with curved forceps to let the animal breathe through the mouth (Lakatos *et al.*, 2006), and the respiration was ensured that the solution was aspirated through deep breaths. The aspirated mice were recovered in the holding tube with a vertical position for 6 minutes, and this is done for all the mice in group II, III and IV during the recovery period (Fig. 4). The consecutive administration to other anesthetized mice could be performed in the course of recovery of another mouse. Therefore, the total experimental time for the whole processing of administration, aspiration and recovery, could be saved, and, for instance, OPA for three mice could be performed within 6 minutes.

Next, the success rate of 95% in oropharyngeal aspiration for EB solution treated mice in group I was investigated through checking the location of EB solution after administration (Table 1). Following OPA, the aspirated EB was found in the lungs of total 19 mice except one mouse. It was considered that the delivery of EB to the stomach in one mouse is the result of short with which the EB solution is not able to reach the lung. This rate was superior to the administration of ITI which could suffer from a high failure rate (Driscoll *et al.*, 2000). To achieve the successful aspiration, it is necessary to keep tongue restrained until at least two deep breaths were completed within 20 seconds.

Fig. 7. The EB localization in the mouse lung after the treatments using OPA.

In group II, the EB was located simultaneously in lung and stomach after 6 minutes following OPA. The reason of these two different delivery locations was considered as the clearance process from mouth and trachea to stomach during the 6 minutes of recovery period.

In the group III, a trace of EB was found after 3 hours in stomach, small intestine and lungs. This recovery period provides additional time for the mechanical clearance of aspirated EB solution from the conducting airways (Rao *et al.*, 2003). Although there were individual differences between the mice, the EB residual migrated evidently from stomach to small intestine by the clearance process.

In the group IV, in many cases, the EB was broadly distributed throughout the left and right lung after 24 hours recovery period (Fig. 6D and Fig. 7). The EB residual was not found in the stomach but most of the residual was in the intestine. According to the previous studies, 11.8~22.5% of beryllium oxide were not found in the respiratory tract after aspiration (Rao *et al.*, 2003). Therefore, we could assume in the present study that the amount of residual EB found in stomach and intestine was over 10%.

In summary, the novel method using syringe pump was developed with a modified slant board for the oropharyngeal aspiration of mouse. This modified method could be simply carried out by one experimenter, and it was a reliable method with the success rate about 95%. However, after exposing the mice to Evans Blue solution, small amount of the EB dye was found in the stomach and the intestine within 24 hours. This oropharyngeal aspiration technique has an advantage to examine the pulmonary effects of respirable particles on the mouse lung. As with inhalation exposure, lung and oropharyngeal clearance will result in gastrointestinal exposure (Keane-Myers *et al.*, 1998; Kimbell 2006). Additional studies are required for better automation of the OPA in future. For instance, the syringe pump in this aspiration method could be controlled by a program which automatically set the dose to be administrated based on the body weight of animals.

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