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Design and validation of a simple device for insufflation of dry powders in a mice model

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ABSTRACT

Delivery of inhalational dry powders (DPs) to the lung of mice is pivotal for pre-clinical pharmacokinetic and pharmacodynamic investigations. Although several devices have been reported, their application is always limited by many factors, including complicated design, high price, commercially discontinued status, as well as requirement of special skills. Here, we have introduced a simple device for non-invasive and precise delivery of DPs in mice. We set up the self-made device using a 20 G cannula tube and a 1 mL syringe. Subsequently, it was validated in terms for proper installation, delivery of dry powder and safety. Taken together, we believe that this device will be helpful in pre-clinical studies, especially in laboratory experiments, for respiratory drug delivery in small animal models.

1. Introduction

Recently, respiratory administration of drug-containing dry powders (DPs) for both local and systemic treatment is gaining popularity. To accomplish drug therapy *via* pulmonary route, drugs need to be delivered either through intratracheal or intranasal route. Delivering drug in the form of DPs to the lung of mice is challenging due to their narrow tracheal tube. Researchers have used various types of devices to deliver drugs to the lung of mice. Some have used commercially available PennCentury™ DP-4 insufflator (Bivas-Benita et al., 2005; Kim et al., 2012; Morello et al., 2009) and others used self-assembled device (Sinha and Mukherjee, 2012) to insufflate DPs in the lung of mice. Insufflating DPs by various techniques allows the drugs to be deposited into the lung ensuring their high bioavailability for local therapeutic effects. To confirm the proper intubation in the lung, various equipments were used by researchers like fibre-optic guided light (Rivera et al., 2005), light-carrying laryngoscope (Molthen, 2006) and water filled in pipe (Watanabe et al., 2009). However, use of the invasive method to deliver drugs into the lung required specific skills and were harmful to animals. In addition, it requires a long lasting anaesthesia which may compromise the circulation and metabolism of the animals (Warheit et al., 2005). Also, the recovery time and risks to the animals following the surgical procedure limit the possibility of multiple-dose drug administration *via* tracheal route. A large number of studies have been done with commercially available PennCentury™ DP-4 insufflator in a mice model. Researchers have given various opinions regarding the use of PennCentury™ DP-4. Some have emphasized that it provides a high level of accuracy in delivering DPs (Kim et al., 2012) while the others have mentioned that the size of delivered DPs gets increased from this device and the required amount of DPs does not reach deep in the lung of mice (Tonnis et al., 2014). Currently, due to the withdrawal of this device from the market, an alternative means for delivery of DPs is urgently required.

Thus, the aim of this work was to develop a convenient, precise and safe DPs delivery device in a mice model as well as characterize it for convenience, accuracy and safety in terms of DPs deposition in the lung. In this study, we have assembled a device very similar to PennCentury™ DP-4 insufflator using a 20 G cannula and a 1 mL syringe, and prepared DiR loaded mannitol DPs by the spray drying technique. An otoscope was used to visualize the tracheal opening of the mice to insert the cannula tube in the wind pipe just above the bifurcation position of the trachea to deliver DPs to the lung. To determine the deposition in the lung of mice, fluorescent imaging technique was adopted. Before insufflation of DPs, safe and optimal air volume was estimated after blowing different volumes of air into the lung of the mice. The air tolerance capacity was characterized by determination of *in vivo*

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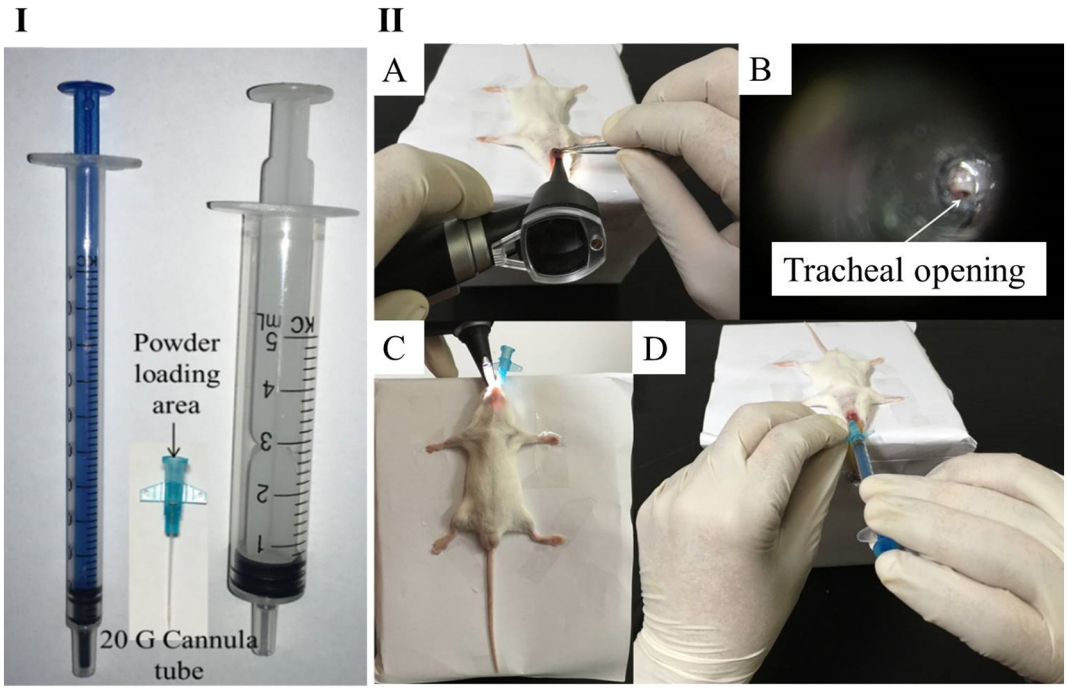

Fig. 1. (I) 20 G cannula tube and plastic syringes (device); (II) Visualization of tracheal opening using otoscope (A); Visualized tracheal opening (marked with white arrow head) (B); Installation of 20 G cannula tube (C); Blowing air to the lung using plastic syringe (D).

abnormalities, such as loss in body weight, physical behaviours of animal after blow of air, and blood coagulation in lung. Moreover, it is noteworthy that the device we designed has been well used in our previous studies (Chaurasiya et al., 2018; Wang et al., 2018), which further introduce the practical use of this device in delivery of DPs with various materials and different sizes.

2. Materials and methods

2.1. Materials

20 G cannula was purchased from BD Venflon, Becton Dickinson Infusion Therapy (Singapore). Plastic syringes (1 and 5 mL) were purchased from Jiangsu Zhang Cheng Yi Liao Qi Xie You Xian Gongsi (Yangzhou, China). Otoscope was purchased from Zumax Medical Co. Ltd. (Suzhou, China). Platform to hold mouse was self-designed. Mouse fixing adhesive tape was purchased from Shandong Hanshifu Glue Co. Ltd. (Shandong, China). Blunt forceps with plastic tip was purchased from Tonglu Wanhe Instruments Co. Ltd. (Tonglu, China). DiR (1,1′-dioctadecyltetramethyl indotricarbocyanine iodide) was obtained from KeyGEN BioTECH (Nanjing, China) and D-mannitol from Sigma-Aldrich (Shanghai, China). Ketamine hydrochloride was purchased from Sigma Aldrich (Shanghai, China).

2.2. Animals

Male BABL/c mice were purchased from Qinglong Mountain Farm (Nanjing, China). All animals were cared in accordance with the guidelines of the National Institute of Health for laboratory use. All animals were housed in groups as per study protocol under 12 h light and dark cycles and fed with normal diet and water *ad libitum*. Before conducting the experiment, all animals were acclimatized for a week. The experiment was conducted with the approval of Ethical Committee on Animal Experimentation of China Pharmaceutical University (Nanjing, China).

2.3. Preparation of DiR-mannitol DPs

DiR-mannitol DPs was prepared by spray drying technique, using a laboratory size Büchi Mini Spray Dryer B-290 (Büchi laboratory-techniques, Switzerland) equipped with a standard size spraying nozzle. An aqueous solution of mannitol (2 g) in 25 mL of water containing DiR solution (640 μg/mL) was spray dried with a pump flow rate of 5 mL/min at an inlet air temperature of 120 °C. The aspirator was set to 100% and the atomizing airflow was adjusted to 500 L_n/h.

2.4. Characterization of physical properties of DPs

The DPs was characterized for particles size, sized distribution, morphology, solid behaviours and aerosol properties. The particle size and size distribution of spray dried powders were determined with Mastersizer (Malvern Instrument, UK) using Scirocco-2000 dry powder sampler. The particle size distribution was determined with the span value according to the following equation, in which the particle distribution was distributed as D_N = 10%, 50%, and 90%. The lower span value determines the narrow particle size distribution.

$$Span = \frac{D_{V,90\%} - D_{V,10\%}}{D_{V,50\%}}$$

The surface morphology of DPs was determined by using a light microscope (Olympus light microscope) and an Ultra-High Resolution Scanning Electron Mircoscope SU8010 (Hitachi high-tech, Japan). Further, the DPs were characterized for bulk and tapped densities, Hausner ratio, Carr's index, and moisture content, and the crystal behaviour of DPs was determined by X-ray powder diffraction analysis.

2.5. Determination of air volume and tolerance capacity of the lung

To determine the air tolerance capacity of the lung, male BALB/c mice (*wt.* 18–20 g) (n = 15) were taken. All mice were grouped into 5 groups (n = 3/group), and they received 0 mL (group 1, control), 0.5 mL (group 2), 1.0 mL (group 3), 2.0 mL (group 4) and 5.0 mL (group 5) of air, respectively. Briefly, all mice were anesthetized with