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Lipopolysaccharide-induced acute lung injury in rats: Comparative assessment of intratracheal instillation and aerosol inhalation

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ABSTRACT

Acute lung injury (ALI) has many possible etiopathologies and is characterized by acute diffuse lung damage with poor prognosis. Lipopolysaccharide (LPS) is widely used as septic model of ALI in pharmacological research. This study compares intratracheal bolus instillation (IT) with dose-adjusted aerosol inhalation (IH) of LPS in Wistar rats using both non-invasive and terminal endpoints. The former comprised exhaled nitric oxide (NOE) and 'enhanced pause' (Penh) both measured in spontaneous breathing conscious rats. Terminal endpoints included lung weights, LDH, protein, total cell counts, and cytodifferentiation in bronchoalveolar lavage (BAL). Measurements were made 1, 3, 7, and 14 days after IT instillation (5 mg LPS/kg body weight) or 6-hour directed-flow nose-only inhalation exposure to respirable LPS-aerosol at 100 mg/m^3 (thoracic dose: 2.6 mg LPS/kg body weight). Controls received saline (IT) or air only (IH). LDH and protein were significantly different from the control in the LPS-IH group (days 1 and 3) with a somewhat inconclusive outcome in the LPS-IT group due to the effects occurring in the control. Total cell counts were equally elevated with similar time-course changes in the LPS-IT and -IH groups. Polymorphonuclear neutrophils (PMNs) were indistinguishable amongst LPS-dosed rats. Again, IT-dosed control rats displayed markedly higher background levels than those dosed by inhalation. Similarly NOE was significantly elevated on post-LPS day 1 as was Penh. In summary, the LPS-aerosol dose delivered by nose-only exposure over 6 h was equally potent as the 2-times higher LPS-IT bolus dose on post-LPS day 1 with somewhat faster recovery thereafter. The climax and discriminatory power of the non-invasive endpoints matched those determined terminally. This supports the conclusion that the pharmacological efficacy and side effects of inhalation pharmaceuticals designed to mitigate ALI can better be identified by LPS-aerosol than by LPS-IT. Non-invasive time-course measurements may deliver apt information both on the efficacious dose as well as the dosing intervals required to maintain the targeted efficacy using a minimum of experimental animals. The outcome of this comparative study supports the conclusion that the inhalation route produces a more uniform type of injury at lower, more meaningful dosages. When designing studies for screening of effective drugs this mode of delivery appears to better approximate the human condition with less dosimetric uncertainty, less experimental variability and better characterization of what was actually delivered to the entire respiratory tract.

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1. Introduction

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) represent a pathophysiological continuum that occurs as a consequence of exposure to direct or indirect pulmonary insult (Rubenfeld, 2003). Pathological changes of ALI/ARDS include diffuse alveolar damage accompanied by neutrophils, macrophages, hyaline membranes and protein-rich edema fluid in the alveolar space (Ware and Matthay, 2000; Goodman et al., 2003). Increased alveolar capillary membrane permeability, due to widespread endothelial and epithelial injury in the pulmonary tissue, was recognized as a predominant feature in all forms of ALI/ARDS (Windsor et al., 1993; Luh and Chiang, 2007).

The rat model devised focuses on the induction of ALI by lipopolysaccharide (LPS) which is widely used as septic model of ALI in pharmacological research. Due to simplicity and expedience, LPS is generally dosed by intratracheal bolus instillation (IT). IT is a

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well-established and widely used method for administering LPS into the rat lungs (Shimizu et al., 2009; da Cunha et al., 2011; Herber-Jonat et al., 2011) but it has a number of limitations, most notably the variability of LPS distribution among different lung lobes. The mode of instillation (supine/prone rat with/without forced ventilation to re-distribute the bolus within the lung, and the bolus volume per se) as well as the acute impairment of the surfactant properties increases both the variability of site-specific pulmonary dosing and effects. The inhalation (IH) of LPS is believed to be better controlled by the analysis of airborne LPS aerosol characteristics and analysis of breathing zone concentrations than bolus IT instillations.

Issues related to the pulmonary toxic dose and the frequency of dosing to attain and maintain pulmonary pharmacological efficacy are particularly challenging for inhalation pharmaceuticals, as drug serum levels may not necessarily mirror local pulmonary drug levels following aerosol delivery. Whatever systemic kinetics has been determined, the mechanisms of drug clearance from the lung may follow different principles. The course taken in this study was to compare whether non-invasive and invasive, terminal methodologies of diagnosis deliver equal information on pulmonary toxicity and whether the methodologies applied can differentiate LPS-induced ALI following IT and IH administration. Hence, the objective of this study was to induce the same degree of LPS-ALI by IT bolus and IH continuous 6-hour nose-only exposure techniques. Acute lung edema and intraluminal inflammation were probed by lung weights, and selected terminal parameters in bronchoalveolar lavage (BAL) fluid and cells. The non-invasive endpoints did not require anaesthesia or any other intervention so that they are amenable to repeated time-course analyses. NO in exhaled breath was believed to be an endpoint that integrates the degree of intraluminal inflammation (Stitt et al., 1997; Kharitonov and Barnes, 2001; Brindicci et al., 2005; Li et al., 2012; Stewart and Katial, 2012) while respiratory function measurements in conscious, spontaneously breathing rats using whole body plethysmography provide a means to monitor rats for extended time periods (in this case approximately 17 h post-LPS dosing). The chosen endpoint ‘enhanced pause’ (Penh) is specifically affected by pulmonary injury affecting the apnea time (pause between end-expiration and following breath). Accordingly, Penh is believed to integrate all effects taking place at parenchymal and stromal levels with resultant change in the control of breathing (Pauluhn, 2004, 2005a,b; Li and Pauluhn, 2010; Li et al., 2011). The replacement of terminal measurements by non-invasive procedures would increase the resolution on time-course analysis with the benefit of less animal usage.

2. Methods

2.1. Test material

Lipopolysaccharides (LPS), from *Escherichia coli* 055:B5, Sigma, Germany.

2.2. Animals, diet, and housing conditions

Healthy male SPF-bred Wistar rats of the strain Hsd Cpb:WU, from the experimental animal breeder Harlan-Nederland (NL), AD Horst, were used. Animals were placed in polycarbonate cages containing bedding material. Both feed and water were given *ad libitum* except during inhalation exposures. At the commencement of study, the mean body weights were approximately 200–230 g. Animal rooms were maintained at approximately 22 °C with relative humidity of 40–60% and a 12-h light cycle beginning at 0600 h. The studies described were in accordance with contemporary, internationally harmonized testing standards/guidelines (OECD, 2009, Available at: http://oberon.sourceoecd.org). The experiments were performed in an animal care-approved laboratory in accordance with the German Animal Welfare Act and European Council Directive 86/609/EEC (Directive 86/609/EEC, 1986) as well the updated Directive 2010/63/EU as of 22 September 2010.

2.3. Experimental design

120 rats in 4 groups were used in the experiments. The 30 rats in each group were divided into 5 subgroups for 4 sacrifice time points and NOE measurement. A dose of 5 mg LPS/kg body weight in aseptic, physiological saline solution (1 ml/kg body weight) was administered intratracheally in the LPS IT group, with physiological saline IT as the control group. The rats in the LPS IH group were nose-only exposed to the aqueous LPS aerosol (concentration 100 mg/m^3) for a duration of 1×6 h. Rats of the IH control group were nose-only exposed to dry, conditioned air. After administration of LPS or saline, rats were similarly anesthetized with pentobarbital (Narcoren®) and exsanguinated. Then the lungs were excised, weighed and lavaged. Six additional rats in every group were used for the NOE measurement at the same time points of 1, 3, 7, 14 days post-administration. For respiratory function, four rats from each group were measured in volume-calibrated whole body, barometric plethysmographs overnight for approximately 17 h post-administration. In an ancillary study time-course analyses following IT instillation of either vehicle or LPS examined the most sensitive respiratory function parameter and time point of analysis.

2.4. LPS intratracheal instillation

Rats were anesthetized superficially with isoflurane (4%) and intratracheally instilled with LPS (5 mg/kg body weight, 1 ml/kg body weight). Briefly, anesthetized rats were placed in a supine position on an inclined platform (approximately 60–70°) with fixation at the incisors to gain free excess to the oral cavity. Metered amounts of LPS were instilled into the trachea (proximal to the bifurcation) by a syringe equipped with a blunt stainless-steel needle which was placed mandarin guided into the trachea via the larynx under laryngoscope control (modified human otoscope). The control groups received aseptic physiological saline. After instillation the rats were ventilated mechanically for about 1 minute in order to equally distribute the bolus within the lung (7025 Rodent Ventilator, Ugo Basile, America, TV: 2 ml, frequency: 75 strokes/min). Once the rats recovered from anesthesia, they were returned to the holding cages and allowed free access to food and water.

2.5. Inhalation exposure system and characterization of atmosphere

A three-segment commercially available (TSE GmbH, Bad Homburg, Germany; www.TSE-Systems.de) aluminum directed-flow nose-only inhalation chamber was used in this study (Fig. 1). Each segment of this two-compartment push-pull inhalation chamber system was suitable to accommodate 20 rats in exposure tubes at its perimeter location. The internal inhalation chamber volume was ~3.8 L per segment. All airflow were monitored and adjusted continuously by means of calibrated and computer controlled mass-flow-controllers. The ratio between supply and exhaust air was selected so that 90% of the supplied air was extracted via the exhaust air and sampling ports. An in-house developed data acquisition and control system was used to control and record all inhalation parameters. The slight positive balance between the air volume supplied and extracted ensured that no passive influx of air into the exposure chamber occurred (via exposure restrainers or other apertures). The slight positive flow balance across an accelerating nozzle and decelerating delivery tube arrangement provides adequate directed ventilation of the exposure port and also minimizes mixing of air of the external chamber with that at the exposure port. The accelerating nozzle of the inner plenum is equidistant to all exposure ports of the chamber. The pressure difference between the inner inhalation chamber plenum and the exposure zone was 0.02 cm H_2O. The exposure system was accommodated in an adequately ventilated enclosure (chemical fume hood). Technical details of this chamber system, including the factors potentially involved with the restraint-related immobilization stress, have been published in detail elsewhere (Pauluhn, 1994; 2005a; Pauluhn and Mohr, 1999, 2000; Pauluhn and Thiel, 2007).

In order to attain a high and temporally stable concentration of LPS, a modified BGI 3-nozzle nebulizer (Type CN-25 MRE, BGI Inc., Waltham MA, USA) was used for LPS aerosol generation (as shown in Fig. 1). The temperature of the nebulizer was maintained at 4 °C using a digitally controlled thermostat to increase stability and to minimize the evaporation of water and changes in solute concentration during the course of aerosolization. Nebulization used conditioned, pressurized air (up to 5 L air/min; LPS solution in water, 3.64%; dispersion pressure approximately 400 kPa). This test atmosphere was entrained into the inhalation chamber with additional dilution of dry air to obtain the targeted breathing zone concentration and particle-size distribution as well as to maintain a minimal air flow-rate of 0.75 L/min per exposure port. The integrity and stability of the aerosol generation and exposure system was monitored real-time using a RAS-2 aerosol photometer (MIE, Bedford, MA, USA). Temperature and humidity measurements in the inhalation chamber were performed by a computerized Data Acquisition and Control System using HC-S3 sensors (Rotronic, http://www.rotronic-usa.com/prod_oem/hc2%20probes/hc2_main. htm). Measured air-flows were calibrated with precision flow-meters and/or specialized flow-calibration devices (Bios DryCal Defender 510; http://www.smglink.com/bios/drycal_defender/drycal_defender.html) and were checked for correct performance at regular intervals. The inhalation chamber was operated in a well-ventilated chemical fume hood. Total mass concentration was determined by gravimetric analysis (filter: Glass-Fiber-Filter, Sartorius, Göttingen, Germany).

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