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Inter- and intrasubject variability of the inflammatory response to segmental endotoxin challenge in healthy volunteers

O. Holz [a,b], L. Tan [c], F. Schaumann [a], M. Müller [a], D. Scholl [c], R. Hidi [c], A. McLeod [c], N. Krug [a,b], J.M. Hohlfeld [a,b,*]

[a] Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM), Clinical Airway Research, Hannover, Germany
[b] Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research, Hannover, Germany
[c] Pfizer Ltd., Clinical Research, Sandwich, Kent, UK

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ABSTRACT

Segmental endotoxin challenge with lipopolysaccharide (LPS) can be used as a pharmacodynamic model to safely induce a transient airway inflammation in the peripheral lung of healthy subjects and to test the anti-inflammatory efficacy of investigational new drugs. In contrast to whole lung LPS challenge only a fraction of the dose is required that can be precisely administered to a specific lung region and a vehicle challenged segment as an intra-subject control can be included. The aim of this study was to assess the intra- and inter-individual variability of the response to segmental LPS challenge for the appropriate design and power calculation of future clinical trials.

Two cohorts with 10 subjects each underwent two segmental LPS challenges within five weeks. The inflammatory response was evaluated in bronchoalveolar lavage (BAL) fluid at 6 (cohort 1) and 24 h (cohort 2) both in the LPS and in a vehicle challenged segment, as well as in plasma for up to 26 h post LPS challenge.

While the cytokine response was more pronounced at 6 h, the influx of neutrophils and monocytes dominated at 24 h; e.g. neutrophils increased from a median (inter-quartile range, IQR) of 0.14 (0.16) and 0.09 (0.08)x10^4 cells/mL BAL fluid at baseline to 10.2 (17.1) and 19.3 (15.9)x10^4 cells/mL 24 h after the two separate challenges. The within-subject variability was higher than the between-subject variability for most of the markers. However, sample size estimations based on the variability of outcome variables found lower or equal numbers with cross-over designs compared to parallel group designs for cellular markers at 24 h and cytokine variables at 6 h.

The segmental LPS challenge model was safe. Future study designs have to balance between burden to the study subjects (4 versus 2 bronchoscopies), variability (within-versus between-subject), and the desired outcome variable (cells versus chemo/cytokine).

1. Background

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation of the airways with increased numbers of neutrophils, monocytes/macrophages and CD8+ lymphocytes [1]. This inflammatory condition is associated with a variety of symptoms (cough, dyspnea, sputum production), progressive decline in lung function, and recurrent exacerbations. While corticosteroid treatment can ameliorate the course of an exacerbation, chronic inflammation often does not respond sufficiently to this treatment. Therefore, there is an unmet need to develop novel anti-inflammatory drugs in COPD.

Drug development of a novel anti-inflammatory agent demands an early proof-of-mechanism in humans, preferably in the diseased population. While airway inflammation in patients with COPD is variable [2] and safety concerns may preclude administration of investigational new drugs in patients with diminished organ

* Corresponding author. Department of Clinical Airway Research, Fraunhofer Institute of Toxicology and Experimental Medicine, Clinical Research Center (CRC), Hannover 30625, Germany.
E-mail addresses: olaf.holz@item.fraunhofer.de (O. Holz), lisa.tan@pfizer.com (L. Tan), frank_schaumann@t-online.de (F. Schaumann), meike.mueller@item.fraunhofer.de (M. Müller), dorotheabeth@yahoo.com (D. Scholl), rabia.hidi@sgs.com (R. Hidi), alison.mcleod@phastar.co.uk (A. McLeod), norbert.krug@item.fraunhofer.de (N. Krug), jens.hohlfeld@item.fraunhofer.de (J.M. Hohlfeld).

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function in early phases of clinical development, disease models in healthy volunteers are warranted. This allows assessing both safety and efficacy to modulate airway inflammation at acceptable risk. In this respect, different pharmacodynamic challenge models have been employed, such as the transient induction of a neutrophilic airway inflammation in healthy subjects by ozone challenge [3] or by challenge with lipopolysaccharide (LPS) [4–6]. The feasibility of these approaches as a proof of mechanism has been demonstrated for a novel CXCR2 antagonist using ozone challenge [7] and for a PDE4 inhibitor using LPS challenge [8,9].

LPS is a major component of the outer membrane of gram-negative bacteria. Chronic exposure to LPS plays a role in the development of dust-related occupational lung disease [10], and LPS is a major component of cigarette smoke [11]. Purified LPS has been safely administered to healthy human volunteers in clinical models of lung inflammation either by inhalation (whole lung deposition) or by segmental challenge [4–6,8,9]. LPS causes an acute, transient systemic and pulmonary inflammatory response [4,6,12,13], the latter being characterised by increased levels of airway neutrophils, CD8+ lymphocytes, monocytes, macrophages and increased concentrations of TNF-alpha (TNFA), interleukin-8 (IL8), and myeloperoxidase (MPO) in sputum or bronchoalveolar lavage (BAL) [6], The pattern of acute lung inflammation is similar to that observed in COPD and therefore LPS challenge has been suggested [14] and employed as a potential clinical model to demonstrate pharmacodynamics of novel anti-inflammatory agents for this disease [8,9,15,16].

Inhaled challenges with LPS have been intensively used by various groups to investigate mechanisms of airway inflammation in humans [4,13,17]. Doses up to 100 μg have been safely applied to humans with doses of about 20 μg LPS as a threshold dose for inducing clinical symptoms and changes in pulmonary function [18]. In contrast, the threshold inhaled dose for inducing activation of inflammatory cells (blood neutrophils) may be less than 0.5 μg LPS [19]. As a pharmacodynamic challenge model, LPS doses between 5 and 30 μg have been used. When using LPS as a challenge agent in clinical trials, many countries require material which has been produced according to good manufacturing practice (GMP). Due to limited resources for GMP grade LPS, there is a need to develop methods with low demand for LPS. In this respect, we have recently shown that controlled inhalation of a low dose of 20.000 Endotoxin Units (EU, 2 μg LPS) can reproducibly induce an inflammatory response [20].

An alternative method that uses low doses of endotoxin is segmental instillation of LPS by bronchoscopy which has first been described by O'Grady and colleagues [6]. Segmental LPS challenge involves flexible bronchoscopy and instillation of LPS into lung segments, with subsequent recovery of inflammatory cells and cytokines by BAL. Although more invasive, it is generally well tolerated and in studies to date without evidence of serious adverse effects or persistent sequelae. Segmental LPS challenge has the advantage that it allows the precise dosing of LPS to a specific lung segment and at the same time the vehicle control administration to a different lung segment, which is not possible in whole lung inhalation challenges.

The aim of the current study was to further characterize the segmental LPS challenge model by examining the intra- and inter-subject variability of inflammatory markers as the basis for the design of future clinical trials. Using a two cohort design, we assessed the inflammatory response at either 6 h or 24 h post-LPS and investigated the variability of the response by repeating the procedure 35 ± 7 days apart. We further aimed to assess the systemic inflammatory response to explore its reproducibility and relationship to BAL fluid markers.

2. Methods

2.1. Study design

In this two-period trial, subjects were allocated to one of two cohorts assessing variability of inflammatory markers either at 6 h (cohort 1) or 24 h (cohort 2) following segmental LPS challenge. Each study participant underwent 8 visits to the study site: In addition to a screening visit at enrolment and a follow-up visit at the end of the trial, each period consisted of a bronchoscopy eligibility visit and 2 bronchoscopy visits. Study periods were separated by 5 ± 1 weeks (Fig. 1).

At screening, informed consent was obtained and inclusion/exclusion criteria (medical history, concomitant medication, electrocardiogram, spirometry, safety labs) were checked. For bronchoscopy eligibility, a coagulation profile and C-reactive protein (CRP) check was performed per period. Subjects underwent a baseline bronchoscopy with BAL and segmental instillation of LPS and saline. Six hours (cohort 1) or 24 h (cohort 2) after the baseline (first) bronchoscopy, a second bronchoscopy with BAL of segments given LPS and saline was performed. Blood samples for biomarker analysis were collected 30 min prior to as well as 1, 3, 5.5, 8, 10, 23.5, and 26 h post LPS challenge. All subjects were contacted within 48 h after discharge from the second bronchoscopy to ensure subjects' safety. Study subjects attended the unit for a follow-up visit 7–14 days after study period 2.

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. Subjects gave their written informed consent. The study was approved by the Ethical Committee of Hannover Medical School.

2.2. Subjects

A total of 37 subjects were screened of which 25 healthy, non-smoking subjects (>5 years non-smoking, <1 pack year) were enrolled in the study (cohort 1: n = 14, cohort 2: n = 11). Volunteers of both genders were aged 18–50 years and had forced expiratory volumes in 1 s (FEV_1) and forced vital capacity (FVC) ≥ 90% of predicted normal with $FEV_1/FVC \geq 0.7$. Twenty subjects (Table 1, n = 10 per cohort) completed all assessments and were included into the analysis of inflammatory biomarkers. Five subjects (4 subjects in cohort 1 and 1 subject in cohort 2) discontinued because of adverse events (AEs) that were not related to study procedures.

2.3. Bronchoscopy and challenge procedure

Bronchoscopies were performed under pre-medication with 200 μg inhaled salbutamol, mild sedation with intravenous midazolam (0.05–0.1 mg/kg) and topical anaesthesia with lidocaine (4% spray, 2% solution, and 2% gel with total lidocaine dose not exceeding 8.3 mg/kg) [9,21]. Subjects also received oxygen during the bronchoscopy and recovery phase. At first bronchoscopy, a baseline BAL of a segment in the left lower lobe was performed with 100 mL of pre-warmed saline. Furthermore, LPS (40 EU Clinical Center Reference Endotoxin (CCRE), National Institutes of Health Clinical Center, Bethesda, MD) per kg body weight in 10 mL sterile saline, equivalent to 4 ng/kg) was instilled into the right middle lobe and 10 mL sterile saline (0.9%) were given into one lingula segment of the contralateral side as control challenge. All subjects were treated with the same batch of LPS. At the second bronchoscopy under identical medication both instilled segments were lavaged with 100 mL saline each.