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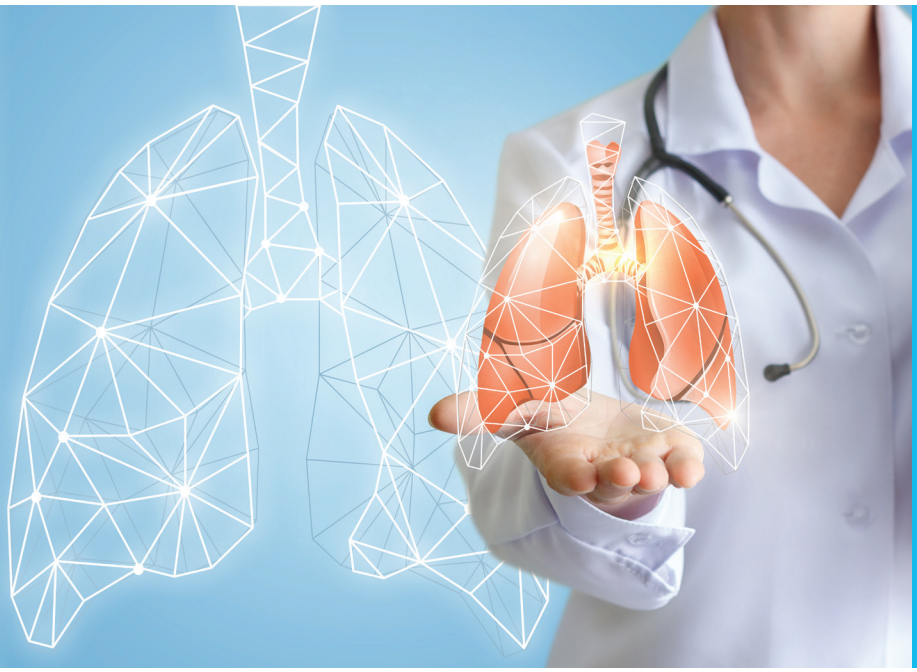
Translating Science to Medicine

Respiratory and Nasal Challenge Studies



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Confirming proof-of-mechanism or target engagement early on in a clinical program is an important milestone for drug development. Typically, drug efficacy is not determined until Phase II when an investigational product is first introduced to a patient cohort. For respiratory indications such as asthma, chronic obstructive pulmonary disease (COPD), chronic cough and allergic rhinitis, effects on disease exacerbations are key study endpoints and can take months to monitor a treatment response. Exacerbations are a worsening of disease condition usually associated with a bacterial or viral infection or environmental pollutant, which can lead to hospitalization and/or respiratory failure. Opportunely, respiratory challenge tests can expedite clinical drug development by exogenously inducing exacerbation-like conditions. Similarly, nasal challenge tests can help mimic the local physiological events occurring during anaphylactic reactions and allergic responses, allowing evaluation of drug effects or impact of nasal congestion on drug absorption following intranasal administration.

During a challenge test, a stimulus (e.g. an allergen, endotoxin, viral agent, or pollutant) is administered to a healthy subject or patient to upregulate systemic and local inflammatory processes in the respiratory system to mimic the diseased state. With a stimulated inflammatory response in a controlled setting, drug efficacy can be evaluated over a shorter period compared to a traditional Phase II study in patients [3]. In addition, a challenge test reduces variability, number of subjects and study costs as the same subject can act as his/her own control in a crossover study design.

The following review will highlight respiratory and nasal challenge tests that employ lipopolysaccharide (LPS), rhinovirus, methacholine, capsaicin, histamine or allergen substrates. Primary outcome of respiratory studies may include changes in forced expiration volume in 1 second (FEV1) by spirometry, effective specific airway conductance by plethysmography, impulse oscillometry (airway resistance) and/or breath nitrogen washout for ventilation heterogeneity. Meanwhile, nasal studies concentrate on assessment of nasal congestion by subject-reported scores and peak nasal inspiratory flow using flow meters in conjunction to nasal drug absorption. Tables 1 and 2 outline the merits and limitations of the respiratory and nasal challenge tests, respectively.

RESPIRATORY DISEASES

ASTHMA: a condition where airways are inflamed and swollen causing shortness of breath and difficult breathing. Asthma affects more than 339 million people globally [1].

COPD: refers to the blockage of airways due to bronchitis and emphysema, resulting in difficulties breathing. COPD is the 4th leading cause of death worldwide [2].

CHRONIC COUGH: a cough that persists for more than 8 weeks. Often related to asthma, allergies, bronchitis or GERD.

ALLERGIC RHINITIS: Commonly known as hay fever and related to seasonal allergies.

LPS CHALLENGE TEST

LPS is an endotoxin that activates toll-like receptor-4 (TLR-4) and induces acute-neutrophil upregulation [4-6], resulting in the elevation of inflammatory cells and cytokines in blood and sputum. In healthy volunteers, LPS can increase a host of cytokines including C-reactive protein (CRP), interleukin 1β (IL-1β), IL-6, IL-8, tumor necrosis factor α (TNFα), myeloperidase, metalloproterinase-9, monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammation protein-1β (MIP-1β). For the challenge test, sterile LPS is administered to a subject with a breath-activated dosimeter. Sputum is induced with increasing concentrations of nebulized hypertonic saline over a five-minute period. Sputum and blood samples are collected before and post inhaled LPS administration. Samples can be analyzed for total sputum cell count, neutrophil, macrophages, leukotrienes counts, neutrophil elastase activity as well as sputum and systemic cytokine concentrations. Following an LPS challenge, serum cytokine measurement allows for evaluation and comparison of the effects of oral and inhaled drugs [7]. The LPS challenge model has been successfully applied for COPD drugs such as prednisolone, fluticasone, PDE4 inhibitors and simvastatin [3]. In a cross-over study, a minimum of two weeks is recommended to "wash out" the LPS-induced airway inflammation.

METHACHOLINE CHALLENGE TEST

Methacholine is a synthetic acetylcholine analogue that acts as an agonist on muscarinic M3 receptors of airway smooth muscle cells. As a direct challenge agent, methacholine causes airflow limitations due to airway narrowing. Methacholine is traditionally used as a high sensitivity substrate for asthma diagnosis and assessment of a treatment, as responsiveness to the challenge test increases with asthma severity. The main outcome of such challenge study is to determine the provocation dose, the concentration that induces a 20% reduction in FEV1 compared to baseline. The test is reproducible and suitable for both adults and children; for these reasons it is recommended by European and American respiratory societies [8]. Higher doses may trigger a response in healthy volunteers [9]. In drug development, the methacholine challenge study has been used to evaluate the efficacy and dose-response relationships of investigational products, demonstrate bioequivalence or compare the components of combination treatments in both healthy volunteers and asthma patients [10, 11].

SPUTUM VERSUS BAL

SPUTUM: mixture of saliva and mucus from the respiratory tract obtained through coughing. Sputum can contain inflammatory cells and mediators for biomarker analysis as well as inhaled drug particles.

BRONCHOALVEOLAR LAVAGE (BAL): an endoscopic procedure capturing cellular and biochemical components from lung fluid during a saline wash. BAL is a minimally invasive technique that can be paired with a biopsy to sample epithelial lining cells.

ACCESS TO PATIENTS

Celerion has a growing database of respiratory patient populations:

Asthma (Mild, Moderate & Severe): > 4200 patients

COPD (Gold Stage 1- 4): > 1400 patients

BRONCHIAL ALLERGEN CHALLENGE

The bronchial allergen challenge can be applied in allergic asthma patients and is used to assess the impact of drugs on inflammatory pathways triggered by an inhaled allergen. The test is a two-step process, first identifying the appropriate substance to induce biphasic, allergic airway reactions, then applying it as a respiratory stimulus to evaluate drug effects. Typically, the allergen causing the greatest skin irritation (skin wheal diameter) following a skin prick test is applied for the airway challenge. Common allergens include dust mite, cat hair, grass and tree pollen. A starting allergen concentration for inhalation is based on the formula derived by Cockcroft [12]. Concentrations are doubled every ±10 minutes and the subsequent, IgE-mediated airway response is repeatedly measured by FEV1 until at least 7 hours after the allergen challenge. An early asthmatic response (EAR) and late asthmatic response (LAR) are usually defined as a reduction in FEV1 of at least 15 or 20% compared to baseline, occurring 0-3 and 3-8 hours after the allergen challenge, respectively. All Celerion clinical research facilities possess the extracted rooms that are required for this type of challenge study.

CAPSAICIN FOR A MODEL OF COUGH

Inhalation of a tussive agents like capsaicin, a pungent component of chili, can be used to trigger coughing in healthy volunteers and chronic cough patients to enable evaluation of pharmacologic responses in early clinical drug development [13]. The tussive agent is delivered in ascending concentrations with a nebulizer. The number of explosive cough sounds occurring within the first 15 seconds after inhalation are then recorded. During cough challenge testing, cough sensitivity is defined as the concentration of capsaicin inducing at least 2 or 5 coughs. Moreover, the maximal cough response (Emax) and the concentration of tussive agent causing 50% of the maximal cough response (ED50) are also assessed.

Table 1. *Merits and Limitations of Respiratory Challenge Tests*

CHALLENGE ASSAY	ADVANTAGES	DISADVANTAGES
LPS	• COPD model • Elicits neutrophilic inflammation • Crossover design allows for reduced number of subjects • Previously used for early phase dose-ranging and POC studies in healthy volunteers and patients	• Not a sensitive model for glucocorticoids • LPS preparation for nebulization requires vigorous mixing and/or specialized containers as LPS can stick to glass tubes [9]
Methacholine	• Diagnostic measurement for asthma hypersensitivity and hyperactivity • Universal guidelines for challenge test [8]	• Diagnosis does not discriminate between asthma, COPD or allergic rhinitis
Allergen	• Well characterized model to assess drug efficacy • Diagnostic measure of occupational asthma	• Allergen depends on subject's skin reaction, could be multiple allergens per study • Specialized extracted rooms
Capsaicin	• Applicable as cough model	• May cause throat irritation

Abbreviations: COPD, chronic obstructive pulmonary disease; LPS, lipopolysaccharide; POC, proof-of-concept

NASAL ALLERGEN CHALLENGE

The nasal allergen challenge model allows the study of the pathophysiology of allergic rhinitis, but in addition it can be utilized in drug development for proof-of-concept studies of novel therapies [14]. Moreover, a nasal allergen challenge in allergic subjects has also been applied as model for nasal congestion to evaluate bioavailability of intranasally administered drugs [15]. The procedure for the nasal

allergen challenge is standardized and reproducibly shown to establish nasal symptoms including rhinorrhea, nasal congestion, nasal itching, and sneezing [16].

Similar to the allergen test described above, a skin prick test is performed to confirm the allergen against which the subject has generated an allergic reaction. Subsequently, a qualifying allergen concentration (QAC) is assessed, which is the concentration establishing a predefined cut-off level of nasal symptoms or decrease in nasal flow within 15 minutes. Ascending concentrations of the allergen are sprayed into the nostril(s) and nasal symptoms (e.g. Total Nasal Symptom Score, TNSS) or nasal airflow (e.g. Peak Nasal Inspiratory Flow, PNIF) are assessed. During the actual nasal allergen challenge, subjects are exposed to a single dose of allergen at the QAC, which is performed at least 15 minutes prior to the administration of study drug.

NASAL HISTAMINE CHALLENGE

When administered to the nasal mucosa, histamine mimics the early phase of an allergen challenge, causing nasal blockage, pruritus, sneezing and rhinorrhea [17]. Histamine, as a vasodilator, increases nasal blood flow, which may impact the transport of drugs administered topically to the nasal mucosa [18]. For these reasons, a nasal histamine challenge can be an alternative to the nasal allergen challenge for proof-of-concept studies for certain novel therapies (e.g. decongestants or anti-histamines), or to evaluate the effect of congestion on intranasal absorption. Compared to the nasal allergen test, advantages of the nasal histamine challenge include lenient inclusion criteria (not limited to allergic subjects), use of a single challenge agent (in contrast to a multitude of allergy-specific allergens), and the lower burden to participants (rapidly resolving symptoms). In addition, a tailored dose may not be required for all purposes. Similar to the nasal allergen challenge, histamine is sprayed into the nostril(s) approximately 5 minutes prior to the administration of study drug and nasal symptoms or nasal airflow are monitored.

RHINOVIRUS CHALLENGE TEST

Up to 60% of exacerbations in COPD are caused by viral infection with human rhinovirus, which leads the common cold [3]. Experimental infection of healthy subjects has been applied in various studies to evaluate the effect of rhinoviral infection on local and systemic innate immunity [19-25]. Others have used this approach with COPD patients [26, 27]. Rhinoviral challenge upregulates IL-6, IL-8, neutrophil and eosinophil responses. The most commonly applied viral serotype is rhinovirus 16 (HRV16) [3], resulting in a lower airway infection upon experimental inoculation. Following baseline assessments, which include collection of a blood sample to determine serum neutralizing antibody titer levels to HRV16 and a throat swab to screen for the presence of other respiratory viruses, subjects undergo a rhinoviral challenge. Subjects are inoculated intranasally with HRV16. A single inoculation of HRV16 can be administered via 4 intranasal instillations (2 per naris) with a cumulative volume of ±1 mL. Following the HRV16 inoculation, subjects will return daily to the clinic for 5 days post inoculation to assess the presence and severity of chest and cold symptoms as well as lung function tests, biomarkers etc. Study drugs can either be administered prior to or after the viral challenge to evaluate their preventative or therapeutic effect. An

advantage of such model is the short-term induction of exacerbations-like events rather than waiting for a patient to naturally catch a cold. A limitation of this method is the variability of infection rates as not all subjects may develop signs and symptoms of the "cold", therefore studies must be adequately powered. In principle, this challenge study may increase the patient burden and, therefore, raise ethical concerns, yet the model has already been tested in COPD patients in pilot studies [27, 28].

Table 2. *Merits and Limitations of Nasal Challenge Tests*

CHALLENGE ASSAY	ADVANTAGES	DISADVANTAGES
Allergen	• Well characterized model of AR • Allows for evaluation of decongestants or anti-allergics [14, 16] • Standardized diagnostic tool for AR	• Allergen depends on subject's skin reaction, could be multiple allergens per study • Inclusion limited to allergic subjects
Histamine	• Straight-forward model mimicking the early phase of allergen exposure [17] • Enables evaluation of decongestants such as anti-histamines • Useful for impact of congestion on nasal drug absorption • Inclusion of non-allergic subjects	• Histamine effect relatively short-lasting (lack of late phase reaction)
Rhinovirus	• "Real world" scenario as a cause of exacerbations	• Variable infection rates • Number of subjects • Ethical consideration for COPD patients

Abbreviations: AR, allergic rhinitis; COPD, chronic obstructive pulmonary disease

SUMMARY

Incorporating respiratory and nasal challenge tests into a clinical trial can expedite drug development by demonstrating target engagement and efficacy prior to patient exposure in a relatively small study in a well-controlled environment. Working with a CRO that understands the advantages and disadvantages for each test is fundamental to study success. Celerion, a full service CRO, has extensive experience with respiratory indication drug development and challenge tests.

CELERION IN-HOUSE EXPERTISE AND SKILLS

- Access to key opinion leaders
  - Membership within the UK's Translational Research Partnership in Respiratory enables faster access to target patient populations, specialists and techniques in Phase I & II studies
- Allergen challenge test rooms
- Spirometry
- Lung clearance index
- Bronchoalveolar lavage
- Body plethysmography
- Fractional exhaled nitric oxide testing
- Cough monitoring
- Lung imaging techniques
  - CT, MRI, PET scans
- Nasal symptom evaluation
- Nasal airflow assessment
- Analytically validated inflammatory cytokines and chemokines biomarkers

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