

Bronchoprovocation Testing

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Abstract Airway smooth muscles of asthmatics tend to be hyperresponsive when provoked. The exaggerated bronchial constriction can be measured by the airflow limitation seen following bronchial provocation. Measuring bronchial hyperresponsiveness by broncho provocation testing is helpful in diagnosing and optimizing therapy. While numerous agents have been used to provoke a measurable airflow limitation, standardized protocols are available for only a few. This article aims to discuss the various methods that have been reported for bronchoprovocation testing.

Keywords Bronchial hyperresponsiveness · Asthma · Bronchoconstriction · Bronchoprovocation

Introduction

Airway smooth muscles of most individuals contract in response to noxious stimuli, resulting in bronchoconstriction. In comparison, the airways of asthmatics are hyperresponsive and the airflow limitation that occurs with a provocative stimulus is exaggerated. Bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma and is attributed to the underlying inflammation in the airways [1]. Measuring bronchial hyperresponsiveness is helpful in the assessment and characterization of airway diseases. Severity of bronchial hyperresponsiveness at baseline may also predict the anticipated response to treatment in asthmatics [2] and can be used as an objective measure in optimizing therapeutic regimen in the long-term management of asthma.

BHR can be diagnosed with bronchoprovocation testing, which involves the application of a stimulus to the airways to

elicit bronchoconstriction, and the resultant airflow limitation is measured. While numerous methods or agents have been used to provoke a measurable airflow limitation in bronchi, standardized protocols are available for only a few. Under standardized protocols, bronchoprovocation testing is an important tool in evaluating airflow obstruction, excluding asthma or differentiating it from other airway diseases, diagnosing occupational asthma, assessing the severity of disease, and determining the response to therapy.

Agents used for bronchoprovocation testing

Numerous agents are known to stimulate bronchoconstriction and have been studied as provocative stimulants (Table 1). These can be broadly categorized as: (1) Selective stimulants and (2) non-selective stimulants.

1. Selective stimulants are used to provoke bronchoconstriction in patients that are suspected to be hyperresponsive to specific agents only. They are particularly useful in evaluating occupational asthma or, in research protocols, aimed at studying the pathophysiology of asthma. Bronchoconstriction seen with selective stimulants may be immunologically or non-immunologically mediated [3]. Immunologic responses occur on exposure and sensitization to agents such as aeroallergens, isocyanates, pollen, and mold. Non-immunologic stimulants include non-steroidal anti-inflammatory drugs (NSAID), acetylsalicylic acid, and food additives. With selective stimulants, the minimum dose required to elicit bronchoconstriction is difficult to predict, and a late response may occur following exposure. Bronchoprovocation testing with selective stimulants is not routinely performed and should be reserved to centers that are experienced and adequately equipped to manage the complications that may arise.
2. Non-selective stimulants may be direct acting or indirect acting. Direct-acting agents bind to agent-specific

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Table 1 Agents used in bronchoprovocation

Selective agents	Non-selective agents
Immunologically mediated	Direct-acting
Aeroallergens	Methacholine
Isocyanates	Histamine
Pollen	Leukotrienes C4, D4, E4.
Mold	Prostaglandin D2
Non-immunologically mediated	Indirect-acting
NSAIDs	Physical stimuli
Salicylates	Exercise
Food additives	<i>Eucapnic voluntary hyperventilation</i>
	<i>Non-isotonic aerosols</i>
	Chemical stimuli
	<i>Adenosine</i>
	<i>Sulfur dioxide/ metabisulfite</i>
	<i>Propranolol</i>
	<i>Tachykinins</i>
	<i>Bradykinins</i>
	<i>Aspirin</i>
	<i>Platelet-activating factors</i>
	<i>Serotonin</i>

receptors on the bronchial smooth muscle cells and cause bronchoconstriction. Methacholine, histamine, leukotrienes C4, D4, and E4 are direct-acting agents that have been studied, and methacholine is the commonly used agent. Indirect-acting stimulants bind to intermediary cells such as inflammatory cells and neuronal cells, causing the release of mediators such as cytokines and neurotransmitters, which in turn cause smooth muscle contraction. Examples of indirect-acting stimulants include chemical agents such as beta blockers, alpha agonists, adenosine monophosphate, prostaglandins, serotonin, acetaldehyde, bradykinins and tachykinins, and physical agents such as cold air, eucapnic hyperventilation, exercise, and non-isotonic aerosols. Non-selective stimulants that are commonly used or being evaluated for bronchoprovocation tests are discussed below.

Bronchoprovocation testing with direct-acting, non-selective stimulants

Methacholine challenge testing

Methacholine is a parasympathomimetic analog of acetylcholine that can be administered as a nebulized aerosol. It binds to receptors on airway smooth muscle cells, mucus glands, and microvasculature and causes bronchoconstriction. Methacholine

challenge testing (MCT) is the most commonly used method of bronchoprovocation testing. It involves administering methacholine in progressively increasing doses while measuring airflow limitation. Guidelines for conducting a MCT, the indications, contraindications, dosing protocols, and objective measures involved are all well established [4, 5].

Indications

MCT is performed when a strong clinical suspicion for asthma exists and spirometry performed before and after administration of a bronchodilator has not established or eliminated the diagnosis. When the pretest probability of asthma is 30–70% [6], MCT has an optimal diagnostic value with a strong negative predictive power to exclude a diagnosis of asthma. BHR can also occur with smoking-induced chronic obstructive pulmonary disease (COPD), congestive heart failure, cystic fibrosis, allergic rhinitis, and bronchiectasis, and MCT cannot distinguish between them. MCT has been used to assess the response to asthma therapy and to tailor the therapeutic regimen accordingly [7]. MCT is also utilized in the evaluation of occupational asthma. Determination of the relative risk of developing asthma in the general population has been suggested, but the routine use in this area has not been recommended.

Contraindications

Conditions that place a patient at increased risk from MCT include a low baseline forced expiratory volume in 1 s (FEV₁) on baseline spirometry, underlying cardiovascular problems such as a heart attack or a stroke in the last 3 months, uncontrolled hypertension (systolic BP>200 or diastolic BP>100), and known aortic aneurysms. With a low baseline FEV₁, there is a risk of potentially serious airway obstruction with bronchoprovocation testing, and according to the current guidelines [4], an FEV₁ of <60% predicted or <1.5 L is a relative contraindication and <50% of predicted or <1.0 L is an absolute contraindication to MCT. Moreover, airway obstruction at baseline renders a positive MCT difficult to interpret. Methacholine is a category C drug and its use in pregnancy and lactation is a relative contraindication. Inability to perform an acceptable quality spirometric maneuvers results in a study that may not be interpretable.

Patient preparation

Bronchoprovocation testing may yield false-negative results if commonly used bronchodilators prescribed to the patients were recently administered. Minimum recommended time interval between the last dose of inhaled bronchodilators and the study is 8, 24, and 48 h for short-acting (albuterol,

terbutaline, isoproterenol, metaproterenol), intermediate-acting (ipratropium bromide), and long-acting (salmeterol, formoterol, tiotropium) bronchodilators, respectively. With leukotriene modifiers, the minimum interval recommended is 24 h. A recent viral infection may result in bronchial hyperresponsiveness and, hence, a false-positive MCT. Inhaled or oral corticosteroids are known to decrease bronchial responsiveness [8] and should be withheld for a few weeks prior to testing. Caffeinated beverages and chocolate are avoided on the day of the test [9].

Dosing protocols

Food and drug administration-approved methacholine (Provocholine) is available in prepackaged, sealed 100-mg vials. Sterile normal saline with or without 0.4% phenol is used as a diluent. Numerous dosing protocols have been published in literature. The two protocols commonly quoted are the 2-min tidal breathing method and the five-breath dosimeter method. In both methods, baseline spirometric measures are made with or without the administration of the diluent only. In the 2-min tidal breathing dosing protocol, 0.03 mg/ml of methacholine is administered via nebulizer for 2 min of relaxed and quiet breathing and FEV₁ is measured 30 to 90 s later. This process is repeated with progressively increasing doses of methacholine (0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/ml) until a 20% decline is seen in FEV₁ from baseline. In the five-breath dosimeter protocol, the patient is instructed to inhale deeply and slowly from the nebulizer containing 0.06 mg/ml of methacholine for 5 s and hold breath at total lung capacity for 5 s. A dosimeter is triggered at the onset of inhalation to deliver the drug. This is repeated five times and then FEV₁ is performed within 30 to 90 s. This process is repeated with progressively increasing doses of methacholine (0.25, 1, 4, and 16 mg/ml) until a 20% decline in FEV₁ is seen. If FEV₁ declines with either protocol, bronchodilator is administered to recover the airflow obstruction to baseline values prior to discharging the patient from the lab. The two different protocols may produce different results [10], and the tidal breathing method is considered to be more sensitive [11].

Endpoint measures

Change in FEV₁ is the commonly used outcome measure in MCT. Spirometry should meet the acceptability and reproducibility recommended by the American Thoracic Society [12]. A decline in FEV₁ by 20% from the baseline measurement within the prescribed dosage schedule is considered a positive test, and the concentration of methacholine that elicits the 20% decline in FEV₁ is represented as PC₂₀. MCT is considered positive when

PC₂₀ is less than 4.0 mg/ml (Table 2). In patients that cannot perform acceptable spirometry, specific conductance (sGaw), a measure of airway resistance (R_{aw}), is used as an outcome measure, and the test is considered positive when a 35% to 45% decline is seen from baseline.

Histamine challenge testing

Bronchoconstriction produced by histamine and methacholine is approximately equivalent at identical doses [13]. Histamine has more systemic adverse effects, and BHR measurements made with histamine are less reproducible [14, 15]. Interpretation of the bronchoprovocation study with histamine is done similar to MCT.

Bronchoprovocation testing with indirect-acting, non-selective stimulants

Indirect bronchoprovocation tests induce bronchial smooth muscle contraction through mediators released by intermediary cells such as the inflammatory cells, epithelial cells, and nerves on airway mucosa. Although most published studies have utilized methacholine or histamine for bronchoprovocation testing, there is an increasing interest lately in indirect challenges. In asthma, bronchial responsiveness to an indirect stimulus is more closely associated with airway inflammation when compared to a direct stimulus [16]. Acute changes in airway inflammation following avoidance of allergens are better measured by bronchial responsiveness to an indirect stimulus [17].

Exercise challenge testing

During exercise, evaporation of water occurs from the airway mucosal surface. Airway cooling and drying exerts a thermal and osmotic effect on the epithelial cells, mast cells, and the sensory nerves. This triggers the release of mediators such as histamine, prostaglandins D₂ and E₂, leukotrienes, and interleukins [18], which results in smooth muscle contraction. Exercise provokes bronchospasm in a majority of asthmatics. The extent of bronchospasm is influenced by the humidity and temperature of the inhaled air during the exercise.

Table 2 FEV₁ baseline measurement

PC ₂₀ (mg/ml) of methacholine	Interpretation
>16	Normal
4.0–16	Borderline BHR
1.0–4.0	Mild BHR (positive test)
<1.0	Moderate to severe BHR

Indications

Exercise challenge testing is used to diagnose exercise-induced asthma or exercise-induced bronchoconstriction (EIB). The test is particularly useful in evaluating patients in whom the presence of EIB would interfere with their line of work (e.g., firefighters, police, military). Exercise testing is also useful in assessing the effectiveness of the treatment prescribed to prevent EIB.

Contraindications

Similar to MCT, airflow limitation at baseline or inability to perform acceptable quality spirometry is considered a contraindication to exercise testing. Patients with unstable cardiac ischemia or malignant arrhythmias should not be tested.

Patient preparation

Patients with EIB may remain refractory to a second challenge for up to 4 h, and therefore, patients should avoid vigorous exercise prior to exercise testing.

Exercise protocols

Acceptable spirometry is obtained for baseline. Exercise is performed on a treadmill or a cycle ergometer while the patient inspires air that is less than 25°C in temperature and less than 50% in humidity. The patient is exercised until the heart rate is 80% to 90% of predicted maximum (calculated as $220 - \text{age in years}$) or the ventilation is 40% to 60% of predicted maximum voluntary ventilation (calculated as $\text{FEV}_1 \times 35$). The target heart rate or ventilation should be achieved within 4 min of exercise to prevent the refractoriness that may develop if exercise is prolonged at submaximal levels. The patient is exercised at target level for at least 4 to 6 min. Exercise is then terminated and spirometry is repeated every 5 min for 30 min.

Endpoint measures

FEV_1 is the primary endpoint measure for exercise challenge testing. A decline of 10% from baseline is abnormal [4], although a 15% decline is considered to be more diagnostic of EIB [19].

Eucapnic voluntary hyperventilation

With eucapnic voluntary hyperventilation (EVH) testing, the patient inhales dry air with 5% carbon dioxide at ventilation equivalent to 30 times the FEV_1 for 6 min. A 10% reduction in FEV_1 from baseline is considered

abnormal. Similar to exercise challenge testing, eucapnic hyperventilation with dry air is more specific and less sensitive than histamine or MCT for the diagnosis of asthma [20, 21]. In comparison to MCT, EVH is better in distinguishing asthma from chronic airflow limitation [22].

Hypertonic aerosols

Bronchial responsiveness to hypertonic saline and dry powder inhalation of mannitol has been studied. Correlation between serum markers of inflammation and bronchial responsiveness to hypertonic saline is better than with MCT [23]. Inhaled mannitol is a portable test that correlates well with exercise challenge testing [24]. Similar to MCT, hypertonic aerosols can be used to elicit bronchial hyperresponsiveness consistent with active asthma or exercise-induced asthma. Testing with hypertonic aerosols is particularly useful in assessing asthmatics preparing to scuba dive [25]. Challenge testing with hypertonic saline helps in the identification of patients with cystic fibrosis with an asthmatic component to their disease [26]. This is important as the inhaled medications administered may be hyperosmolar and may exacerbate bronchoconstriction [27]. Hypertonic saline is useful in bronchoprovocation testing in pregnancy when pharmacologic agents may be contraindicated.

Inhaled AMP

Adenosine 5'-monophosphate (AMP) is known to stimulate the release of histamine from mast cells [28]. Atopic individuals are more responsive to inhaled adenosine and AMP than to methacholine [29]. Bronchoconstriction induced by AMP is an index of mast cell priming. Nasal challenge with AMP elicits rhinitic symptoms and an increase in histamine levels in the lavage fluid, with greater response seen in atopic individuals when compared to non-atopic individuals [30]. In patients with allergic rhinitis, sputum eosinophilia correlates better with bronchial hyperresponsiveness to AMP than to methacholine [31]. Adenosine challenge tests are helpful in distinguishing pediatric asthma from pediatric chronic obstructive diseases such as cystic fibrosis, bronchiectasis, ciliary dyskinesia, and bronchiolitis obliterans [32]. Among adult non-smokers, adenosine challenge testing is useful in distinguishing between asthma and chronic obstructive pulmonary disease [33]. In patients with asthma, PC_{20} AMP correlates better with eosinophilic airway inflammation than PC_{20} methacholine [16]. PC_{20} AMP is more sensitive to changes in acute airway inflammation compared to PC_{20} methacholine [34]. Treatment with inhaled corticosteroids improves responsiveness to AMP to a greater extent when compared to responsiveness to methacholine [35]. The aforementioned

findings indicate that adenosine challenge test may become an important tool in distinguishing asthma from chronic obstructive lung diseases and monitoring disease activity.

Aspirin

Identification of aspirin or non-steroidal anti-inflammatory drug intolerance among asthmatics has relied on challenging the patients with incremental doses of oral aspirin [36]. Oral provocation tests are time-consuming and the safety is of concern. Inhalational challenge with lysine aspirin has been used to diagnose aspirin-induced asthma [37]. Bronchoconstriction occurs within 20 to 30 min following the inhalational challenge, with lesser systemic effects and can easily be reversed [38]. Following the test, the patients are refractory to stimulation with aspirin or NSAIDs for 2 to 5 days and the test cannot be repeated for at least a week.

Propranolol

Propranolol inhalational tests are thought to be more specific than methacholine or histamine in diagnosing asthma [39, 40]. However, bronchoconstriction following propranolol inhalation is less well tolerated and is weakly reversed by inhaled beta agonists or anticholinergics.

Metabisulfite and sulfur dioxide

Inhalation of gaseous sulfur dioxide, which is a common air pollutant, or aerosolized sodium metabisulfite produces bronchoconstriction, and a challenge test utilizing them is useful in distinguishing asthma from COPD [41]. The data available are limited and need further investigation.

Tachykinins and bradykinins

Tachykinins are neuropeptides released from unmyelinated sensory nerve fibers of lower airways and various non-neural cells (endothelial cells, eosinophils, macrophages) and are released in response to allergen exposure and result in smooth muscle contraction [42, 43, 44]. Bradykinins are vasoactive peptides, which are breakdown products formed during an inflammatory process. In patients with asthma, there is a dose-dependant airflow limitation following exposure to inhaled bradykinins [45, 46, 47]. Using tachykinins and bradykinins for bronchoprovocation tests is limited to research purposes at this time owing to the high cost involved.

Conclusion

Bronchoprovocation testing with non-selective agents is widely performed in the evaluation of patients with

symptoms suggestive of asthma but with normal spirometry. The commonly used direct acting agent is methacholine and indirect method is exercise challenge test. Both these tests have been evaluated extensively and guidelines have been established to standardize the test and to interpret the results. While direct-acting agents are useful in eliminating the diagnosis of asthma, a positive test has poor specificity. Lately, there is an increasing interest in indirect-acting stimuli and studies have suggested that provocation tests with indirect stimuli are more specific for asthma and correlate better with airway inflammation. As a result, bronchoprovocation with indirect-acting stimuli may gain wider acceptance in the future for the diagnosis of asthma and for long-term monitoring of disease as a surrogate marker of inflammation.

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