ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, 13 SECTIO D 2002

Pulmonary Department, University School of Medicine, Lublin
Katedra i Klinika Chorób Płuc i Gruźlicy Akademii Medycznej w Lublinie

JANUSZ MILANOWSKI

Significance of bronchial challenge testing in pulmonary medicine

Znaczenie prowokacyjnych testów oskrzelowych w diagnostyce chorób układu oddechowego

BRONCHIAL CHALLENGE TESTING

Bronchial inhalation challenge tests are used as a means of studying asthma and different pulmonary diseases at the experimental and human level for investigative and diagnostic purposes. They have contributed significantly to our understanding of the pathophysiology and the pathogenesis of asthma and to our understanding of mechanisms of antiasthmatic drug therapy.

In terms of their clinical application, bronchial challenge tests may be categorized as follows: (1) tests of *nonspecific airway responsiveness*, and (2) tests of *specific airway responsiveness*. Specific airway responsiveness pertains to reactions induced by specific agents, that is, those that are capable of sensitizing airway tissue, such as allergens and certain occupational agents. Nonspecific responsiveness, by contrast, describes the state of bronchial reactivity to different stimuli, such as such as methacholine and histamine. The present discussion considers both types of challenge tests and focuses on the technical aspects of challenge procedures, the characteristics of airway reactions induced by controlled provocation, and the uses and significance of these tests.

TESTS OF NONSPECIFIC AIRWAY RESPONSIVENESS

Abnormal nonspecific airway responsiveness (hyperresponsiveness), is a characteristic feature of bronchial asthma [2]. Airway hyperresponsiveness may manifest clinically as a cough and bronchospasm with exposure to inhaled irritants, with physical exertion, or with mechanical stimulation of the airways. In the laboratory abnormal responsiveness is recognized by an increased bronchoconstrictor response to inhaled bronchoactive agents or to physical stimuli, such as exercise or hyperventilation. The most widely used method for assessing nonspecific responsiveness involves aerosol administration of pharmacologic agents such as histamine and methacholine.

PHARMACOLOGIC AGENTS AND TECHNICAL PROCEDURES

Pharmacologic agents are delivered in incremental concentrations until a desired pulmonary function change is observed. The initial concentration of histamine or methacholine should be less than 0.1 mg/ml in order to avoid severe reactions.

Methacholine chloride and histamine acid phosphate may be diluted in either saline or buffered saline. When stored at 4°C, both agents maintain stability for several months [16, 14]. Although histamine and methacholine differ in molecular weight, both agonists give similar results. The challenge begins by measuring the response to a saline control aerosol. Responses to subsequent provocative agent concentrations are expressed by the pulmonary function change relative to the saline value. The dose of agonist is expressed by one of several ways: (1) as the concentration inhaled (mg/ml), (2) as the cumulative amount of agonist delivered from the nebulizer, or (3) as cumulative inhalation breath units. An *inhalation breath unit* has been defined as the equivalent of one breath of a concentration containing 1 mg/ml [4]. The most common variable used to express nonspecific responsiveness is the dose causing a 20% fall in FEV_1. Depending on the expression of dose, this variable is presented as the PC_{20}–FEV_1 (mg/ml), the PD_{20}–FEV_1 (micromoles), or the PD_{20}–FEV_1 (breath units).

Responses to pharmacologic agonists may be altered by different stimuli: medications, baseline airway caliber, respiratory infections, and exposure to allergens and chemical sensitizers [5].

INTERPRETATION OF PHARMACOLOGIC CHALLENGE TESTS

In epidemiologic and clinical research studies, pharmacologic challenge tests have been used most widely as a diagnostic test for asthma. Challenge studies in asthmatics showed that an abnormal histamine or methacholine response is a sensitive test with a high negative predictive value. The degree of methacholine or histamine responsiveness has been shown to correlate with asthma severity. The level of responsiveness also correlates with diurnal fluctuations in peak expiratory flow

A number of studies have demonstrated an association between abnormal airway responsiveness and other respiratory conditions including cystic fibrosis, viral respiratory infections, exposures to allergens [5, 1] and diseases characterized by chronic airflow limitation such as chronic obstructive pulmonary disease. Changes in responsiveness that occur with acute inflammatory conditions are usually transient and may return to normal in several weeks. Abnormal responsiveness associated with chronic bronchitis tends to be fixed and correlates with the level of pulmonary function impairment. For this reason, pharmacologic challenge tests cannot be used to discriminate between asthma and diseases characterized by nonreversible airflow limitation in patients with abnormal spirometry. In asthmatics, increased responsiveness is often demonstratable even when spirometry is normal. For this reason challenge testing is used in the evaluation of patients with normal spirometry who demonstrate respiratory symptoms. In these patients provocation testing may be very useful in defining the cause of symptoms and establishing an appropriate treatment.

Pharmacologic challenge tests are generally safe. Severe symptomatic reactions may occur, in some patients with high level of bronchial hyperreactivity. For this reason, it is recommended that airway challenge tests should be performed by experienced physicians who are well trained in this area.

TESTS OF SPECIFIC AIRWAY RESPONSIVENESS

A different specific sensitizing agents have been used in bronchial challenge testing for investigative and clinical purposes. Many of these agents are well-defined aeroallergens. Others are poorly characterized because of their complexity, such as organic antigens causing hypersensitivity pneumonitis, and a variety of occupational chemicals causing asthma [12]. Bronchial challenge techniques can vary according to the particular agent used, especially occupational agents. Almost any allergen capable of penetrating the lower respiratory tract can be used for bronchial provocation. The allergenic substance should contain particles of respirable dimensions, usually smaller than 10 μ in diameter. Allergens used for bronchial challenge should also be suitable for skin testing, since the allergen dose delivered to the airways is usually determined by the skin sensitivity of the patient. Aqueous allergenic extracts are most popular. Aerosolized powders and pollen fragments can also be used provided that they are administered in small enough particle size [15]. Allergens should be standardized for potency and purity should be used, although in reality few allergens are so characterized.

Most commercially available allergens are obtained as lyophilized extracts or as concentrated solutions. When stored at –20°C, lyophilized extracts or their concentrates retain potency for an indefinite period. When reconstituted, however, extracts tend to degrade with time.

The most commonly used method of allergen challenge testing involve: a graded dosing technique, in which incremental allergen concentrations are given sequentially until the desired pulmonary response is achieved. For reasons of safety, proper selection of the initial allergen concentration is the most important steps of the procedure. Initial concentrations that are too high may lead to severe reactions, whereas too low concentrations may lead to prolongation of the challenge procedure. There exist no simple guidelines for recommending starting concentrations, because of variation in patient sensitivity, as well as differences in potency and purity among different allergens. As a rule, the selection of an initial concentration is guided by the skin test sensitivity of the patient. Previously published guidelines have recommended as a starting concentration that which produces a 2 + (larger than 5 mm wheal) reaction after intradermal injection [4, 6]. Although this approach is safe, it may result in an unnecessarily prolonged test in some patients, often taking as long as 2 to 3 hours to provoke a mild asthmatic response.

Some data indicate that the response to inhaled allergen is determined not only by the level of allergic sensitivity, but also by the level of nonspecific airway responsiveness. Thus the response to allergen is viewed as a product of the quantity of mediators

liberated, as well as the airway responsiveness to mediators. Subjects with relatively low levels of nonspecific airway responsiveness may respond to inhaled allergen, provided that they are highly allergic and a high enough dose of allergen is administered. Conversely, subjects with exaggerated non-specific airway responsiveness can also demonstrate reactions, although they possess only a modest degree of allergic sensitivity.

AEROSOL DELIVERY SYSTEMS

A variety of aerosol delivery systems are used for allergen challenge testing. Although different systems vary with regard to the type of equipment used and the pattern of aerosol inhalation, each is designed to optimize reproducible delivery to the lung so that an inhaled allergen dose can be calculated. In general, the dose delivered to the lower respiratory tract depends on the output characteristics of the nebulizer and several physiologic factors, such as pattern of inhalation and airway patency [7]. It is recommended that nebulizers generating aerosols with a particle size range between 1 and 5 microns be used in order to minimize deposition of large particles in the mouth, oropharynx, and upper airway, as well as loss of aerosol due to exhalation of particles less than 1 micron. The distribution of aerosols within the lung is influenced by inspiratory volume and flow rate, lung volume at the start of aerosol inhalation, and breath-holding time.

PHYSIOLOGIC MONITORING

Physiologic responses to inhaled aerosols can be measured by a variety of pulmonary function tests. The choice of a pulmonary function measurement is based primarily on the purpose of the investigation. Studies examining preferential effects of an aerosol on peripheral versus central airways, for example, may require the use of tests designed to discriminate between large and small airways obstruction, such as flow volume curves breathing air and a helium-oxygen mixture. The best way is to use a battery of physiologic tests designed to assess different aspects of lung function . In practice, experience has shown that simple spirometric tests are usually adequate. In particular, the 1-second forced expired volume (FEV_1), the volume of gas expired in the first second of a maximum forced exhalation after full expansion of the lungs, is perhaps the most commonly used variable in evaluating inhalation challenge responses. Because FEV_1 measurements are widely used in clinical practice, challenge results are easily analized. In addition, the FEV_1 is among the most reproducible of lung function tests; the coefficient of variation for replicate tests in trained subjects is less than 3%. Further advantages of the FEV_1 are ease of performance and the requirement for inexpensive equipment. Measurements of peak expiratory flow rate (PEFR) are also commonly used and, in my experience, changes in PEFR closely parallel changes in FEV_1. Reliable and inexpensive devices are also available for PEFR measurements, and because such devices are lightweight and portable, they can be used by the patient to assess lung function changes after leaving the laboratory.

ALLERGEN CHALLENGE PROCEDURES

The most common method currently used is a graded-dose challenge protocol, which is recommended for safety and for establishing antigen dose-response relationships. The procedure begins with the administration of a control aerosol (usually saline). Pulmonary function tests, usually the FEV_1 are measured before and after the control aerosol and after each dose of allergen. In general, subjects demonstrating less than a 10% fall in FEV_1 with the control aerosol are considered suitable for challenge. Allergen extracts are administered in two-fold concentration increments until the desired pulmonary function end point is achieved. Most allergen extracts provoke either an immediate or a combination of an immediate and delayed obstructive response. In the immediate IgE-mediated reaction, peak pulmonary function alterations generally occur within 10 to 12 minutes after aerosol administration, and such changes may persist longer, with a gradual return toward prechallenge pulmonary function. Accordingly, incremental allergen concentrations are ideally administered at 12- to 15-minute intervals, and pulmonary function measurements are made between 10 and 15 minutes after challenge.

FACTORS INFLUENCING ALLERGEN CHALLENGE RESPONSES

The response to inhaled allergen is determined by the level of nonspecific airway responsiveness, but factors that alter the latter may significantly influence the results of challenge tests. For example, increased histamine and methacholine responses have been found after prior exposure to allergens and occupational, sensitizers, after viral respiratory tract infections, and after exposure to oxidizing pollutants [5]. The potentiation of nonspecific airway responsiveness after viral infection may persist for as long as 7 weeks.

A number of medications also alter allergen responsiveness. The inhibition of the early airway response to allergen have been reported with H_1 antagonists. Tricyclic antidepressent agents also possess potent anti-H_1 activity and may alter allergen responses.

Inhaled beta-adrenergic agents are the most potent inhibitors of the early airway response to inhaled allergen. The effects of methylxanthines remain somewhat controversial.

Antiallergic drugs, whose mechanism of action is to inhibit mediator release from mast cells, inhibit allergen responses in most patients. Cromolyn sodium can block the immediate airway response to allergen challenge after a single inhaled dose. Moreover, cromolyn sodium has been shown to significantly inhibit late airway responses to allergen. The effects of corticosteroids on immediate responses to allergen, on the other hand, are less clear. Anticholinergic agents may inhibit immediate responses to allergen in some subjects, although the effect is small and of questionable significance.

CHARACTERISTICS OF ALLERGEN RESPONSES

The typical reaction to inhaled allergen is characterized by an immediate (early) obstructive response that develops within 10 to 20 minutes or a combination of an immediate response and a late reaction that develops within 3 to 8 hours after challenge. The pathophysiologic alterations that occur in the immediate response are similar to those that occur during acute spontaneous attacks of asthma. Reductions in vital capacity, increases in residual volume and functional residual capacity, and abnormalities of all pulmonary function measurements that reflect airflow limitation are characteristically demonstrated. Reversible increases in total lung capacity may occur after controlled allergen challenges as well. Other alterations reported with allergen challenge include hypoxemia, increases in physiologic dead space, and increases in pulmonary artery pressure. In general, immediate airway reactions resolve spontaneously within 1.5 to 3 hours. Reactions characterized by a 20% to 30% reduction in FEV_1 are usually safe and well tolerated by the patient. Moreover, immediate reactions generally are easily reversed by the inhalation of a beta-adrenergic agonist.

Late airway reactions are also characterized by an obstructive process, and thus they differ from the delayed Arthus-type reactions typical of hypersensitivity pneumonitis where there is a predominant restrictive impairment [17]. Allergen-induced late airway reactions are usually of longer duration and more resistant to reversal by beta-adrenergic agonists than early airway reactions. In fact, asthma symptoms may persist for several weeks after allergen challenge in subjects experiencing late reactions.

Inhibition of the late airway reaction has been demonstrated with corticosteroids and cromolyn sodium. Cromolyn sodium blocks both early and late allergen reactions, whereas corticosteroids tend to inhibit only the late reaction. Beta-adrenergic agonists, given prior to challenge, inhibit the early reaction but have no effect on the late reaction.

USES AND INTERPRETATION OF ALLERGEN CHALLENGE TESTS

Allergen challenge tests have been employed most productively as an investigative tool in studies of the pathophysiology and mechanisms of asthma and more recently in studies of the effects or new antiasthmatic compounds [9]. The clinical applications of allergen challenge tests are less well established. Such tests appear to be of diagnostic value as they offer objective information concerning the etiologic role of specific agents in hypersensitivity diseases of the lower respiratory tract. In practice, however, this theoretical advantage has proven useful in limited clinical situations, primarily those involving cases of suspected occupational asthma or hypersensitivity pneumonitis, where the historical relation between exposure and symptoms is unclear and where the suspected agent is unsuitable for diagnostic skin testing. Because many occupational sensitizing agents are poorly characterized, quantitative information concerning exposure levels is often difficult to obtain. Challenge procedures performed

with occupational allergens or other unusual sensitizing agents are therefore often carried out in the native environment or the workplace, where reaction severity can be predicted. Laboratory challenges may also be performed, but in this case the exposure protocol is designed to closely simulate natural exposure. These challenges should be performed by physicians experienced in the use of a particular agent.

REFERENCES

1. *Boulet L.P., Cartier A., Thomson N.C., et al.*: Asthma and increases in non-allergic bronchial responsiveness from seasonal pollen exposure. J. Allergy Clin. Immunol., 1983; 71: 399.
2. *Boushey H.A., Holtzman M.J., Sheller J.R., Nadel J.A.*: Bronchial hyperractivity. State of the art. Am. Rev. Respir. Dis., 1980; 121: 389.
3. *Cartier A., Thomson N.C., Frith P.A., et al.*: Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J. Allergy Clin. Immunol., 1982; 70: 170.
4. *Chai H., Farr R.S., Froehlich L.A., et al.*: Standardization of bronchial inhalation challenge procedures. J. Allergy Clin. Immunol, 1975; 56: 323.
5. *Cockroft D.W., Ruffin R.E., Dolovich J., Hargreave F.E.*: Allergen-induced increase in non-allergic bronchial reactivity. Clin. Allergy, 1977; 7: 503.
6. *Cropp G.J.A., Bernstein I.L., Boushey H.A. Jr., et al.*: Guidelines for bronchial inhalation challenges with pharmacologic and antigenic agents. ATS News, 1980; 6 (2): 11.
7. *Dolovich M.B.*: Technical factors influencing responses to challenge aerosols. In: Hargreave F.E., Woolcock A.J. (editors): Airway responsiveness. Mississauga 1985, Astra, p.9.
8. *Fish J.E., Menkes H.A.*: Airway reactivity: role in acute and chronic disease. In: Simmons D.H. (editor): Current pulmonology, vol. 5, New York, 1984, John Wiley & Sons, p. 169.
9. *Hargreave F.E., Fink J.N., Cockroft D.W., et al.*: Report of The American Academy Of Allergy and Immunology Task Force on guidelines for clinical investigation of nonbronchodilator antiasthmatic drugs: the role of bronchoprovocation. J. Allergy Clin. Immunol, 1986; 78 (suppl.): 517.
10. *Howarth P.H., Durham S.R., Lee T.H., et al.*: Influence of albuterol, cromolyn sodium and ipratropium bromide on the airway and circulating mediator responses to allergen bronchial provocation in asthma. Am. Rev. Respir. Dis., 1985; 132: 986.
11. *Marsh W.R., Irvin C.G., Murphy K.R., et al.*: Increases in airway reactivity to histamine and inflammatory cells in bronchoalveolar lavage after the late asthmatic response in an animal model. Am. Rev. Respir. Dis., 1985; 131: 875.
12. *Milanowski J., Brzeski Z., Sodolski W., Skórska C.*: Problemy diagnostyczne oraz orzecznicze alergicznego zapalenia pęcherzyków płucnych u rolników i hodowców drobiu. Przegl. Lek., 2001; 58: 42.
13. *Pepys J., Hutchcroft B.J.*: Bronchial provocation tests in etiologic diagnosis and analysis of asthma. Am. Rev. Respir. Dis., 1975; 112: 829.
14. *Pratter M.R., Woodman T.F., Irwin R.S., Johnson B.*: Stability of stored methacholine chloride solutions. Am. Rev. Respir. Dis., 1983; 126: 717.
15. *Rosenberg G.L., Rosenthal R.R., Norman P.S.*: Inhalation challenge with ragweed pollen in ragweed-sensitive asthmatic. J/ Allergy Clin. Immunol., 1983; 71: 302.
16. *Rosenfeld M.M., Juniper E.F., Hargreave F.E.*: Stability of histamine acid phosphate solutions. J. Allergy Clin. Immunol., 1984; 73S: 151A.
17. *Schlueter D.P.*: Response of the lung to inhaled antigens. Am. J. Med., 1974; 57: 476.

STRESZCZENIE

W pracy omówiono znaczenie inhalacyjnych testów prowokacji wziewnej w diagnostyce chorób układu oddechowego: astmy oskrzelowej, alergicznego zapalenia pęcherzyków płucnych. Opisano techniki wykonywania testów nieswoistych i swoistych, zwracając szczególną uwagę na interpretację wyników badań i ich znaczenie kliniczne.