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Bronchial provocation tests: The rationale for using inhaled mannitol as a test for airway hyperresponsiveness

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The use of histamine and methacholine is well established for identifying airway hyperresponsiveness (AHR) but the AHR to these agents is not specific for asthma diagnosis. Further, these agents do not identify or exclude exercise-induced asthma (EIA) so they are inappropriate for some occupational and sporting assessments. Measurement of AHR by pharmacological agents has other limitations in that a positive response does not necessarily identify a person who will respond to inhaled steroids and responses do not differentiate between doses of steroids. As most asthmatics remain hyperresponsive to these agents after treatment they have not been useful for guiding steroid dose reduction. Bronchial provocation tests (BPTs) with physical stimuli such as exercise, eucapnic voluntary hyperpnea and hypertonic saline have provided useful information on presence and severity of asthma and EIA. These tests however, can be time consuming and require more resources compared with the pharmacological tests. To simplify testing, a challenge has been developed that uses a dry powder of mannitol administered from a simple hand-held device. The mannitol is given in increasing doses from capsules containing from 5 mg to 40 mg. Mannitol responsiveness identifies people with EIA and those who will respond to inhaled steroids. Mannitol responsiveness is reduced following treatment with inhaled steroids, and some subjects become unresponsive within 6 to 8 weeks. Responsiveness to mannitol can be used to predict risk of exacerbation during back titration of steroids. Should this BPT become more readily available it would be the first to provide a common operating standard for use in the laboratory, office, or field.

Key words: bronchial provocation tests; asthma; osmotic aerosols; histamine; methacholine; exercise; mannitol

Introduction

Airway hyperresponsiveness

Asthma is a disease of the airways that makes them prone to narrow too much and too easily in response to a wide variety of provoking stimuli [1]. This variable airflow limitation is a hallmark of asthma, and one approach to its measurement is to demonstrate improvement of the forced expiratory volume in one second (FEV_1) after inhalation of a standard dose of $beta_2$ agonist [2]. Variable airflow limitation can also be reproduced in a lung function laboratory through the administration of standardised bronchial provocation tests (BPTs) that assess airway responsiveness. In recent times, BPTs in controlled settings are becoming more widely used to investigate if the airways narrow too much and too easily, and if there are changes in sensitivity in response to treatment [3]. These tests, while largely confined to Pulmonary Function Laboratories, have also been adapted for use in the field to assess prevalence of asthma in different communities, e.g. school and workplace.

Airway hyperresponsiveness (AHR) is associated with self-reported respiratory morbidity and clinically defined asthma [4–7]. Epidemiological studies have shown that AHR is increased in atopic individuals [5, 7, 8], is affected by airway calibre [9], is prevalent in winter sports athletes like cross-country skiers [10] or ice hockey players [11], is a risk factor for reduced growth in FEV_1 in children

Abbreviations

AHR	airway hyperresponsiveness
BPT	bronchial provocation test
EIA	exercise-induced asthma
EVH	eucapnic voluntary hyperpnea
PD	provoking dose
FEV_1	forced expiratory volume in one second

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[12], and is correlated with exhaled nitric oxide in atopic children [13]. It is now recognised that the measurement of AHR is not only useful in diagnosing asthma [14] but also in monitoring response to therapy in asthmatic patients [15–17]. Asthma control is better when a decrease in AHR is demonstrated. Further, a measurement of AHR can be used to predict asthma exacerbation following reduction of inhaled corticosteroids [18].

Bronchial provocation tests

There are two categories of bronchial provocation tests, "direct" and "indirect". The "direct" category includes the pharmacological agents histamine and methacholine (an analogue of acetylcholine), and were first used more than 50 yrs ago [19]. These agonists cause airway narrowing by acting "directly" on their respective receptors on bronchial smooth muscle to cause contraction. While recognised as sensitive tests for identifying AHR to a single "mediator", it is now appreciated that the AHR identified is not specific to the diagnosis of asthma. Healthy, non-asthmatic persons [4, 7, 20], persons with chronic obstructive lung disease [21, 22], and smokers can also have airway hyperresponsiveness to these agents [23]. While pharmacological agents are considered sensitive for identifying asthma in patients with clinically recognised asthma referred to a laboratory [24], they are less sensitive for detecting known asthmatic subjects in field studies [4, 25]. The reason for this may simply relate to only one "mediator" being investigated during a pharmacological challenge, whereas many endogenously-released mediators are involved in the airway narrowing of asthma.

The second category is known as "indirect" challenge tests and include the physical (as in non-chemical) stimuli such as exercise, hyperpnea of dry air, distilled water, hypertonic saline and mannitol, and the pharmacological agent adenosine monophosphate. These stimuli are thought to cause airway narrowing "indirectly" by releasing a wide variety of mediators of bronchoconstriction from inflammatory cells within the airway. These mediators then act on their specific receptors on bronchial smooth muscle to cause contraction, and airway narrowing is a consequence of this [26]. The physical agents are more specific for identifying asthma [21, 27, 28], though less sensitive for identifying AHR in a laboratory population [29]. However, the sensitivity of the "direct" and "indirect" tests to identify AHR in the field is very similar [25, 30]. Persons who do not report asthma do not usually respond to "indirect" stimuli [31, 32]. Persons with very mild asthma who are well controlled on inhaled corticosteroids may not respond to "indirect" tests, and the control of exercise-induced asthma by inhaled corticosteroids is a good example of this [33].

The utility of bronchial provocation tests in assisting a diagnosis of asthma, monitoring asthma therapy, and assessing asthma prevalence in the community has led to a need for standardised tests that are portable, rapid to perform, and inexpensive. There are many different protocols used for administering the many "direct" and "indirect" BPTs available [3], and this has been one of the problems in implementing these tests. A further problem has been the lower specificity of pharmacological tests to identify active asthma. A new test that involves the inhalation of a dry powder of mannitol provides the potential for a common operating standard. Further, it provides an opportunity to identify subjects with exercise-induced asthma (EIA) and active airway inflammation responsive to inhaled steroids and other drugs used in the treatment of asthma.

The aims of this review are to give an historical perspective of the development and use of "indirect" challenge testing using physical stimuli, and to provide a rationale for using mannitol for testing to evaluate airway hyperresponsiveness.

Evolution of the indirect BPTs using physical stimuli

In the early 1960s Jones et al. reported that children with asthma had characteristic changes in FEV_1 in response to vigorous exercise [34]. Jones et al. concluded that the post-exercise fall in FEV_1 is so constant in the asthmatic that a failure to demonstrate it should lead to reconsideration of the diagnosis or of the technique of the test [34]. This led to the development and standardisation of exercise testing to identify exercise-induced asthma (EIA) [35]. The similarity in the physiological changes that occurred with EIA (hypoxaemia and hyperinflation) and other forms of provoked asthma meant that exercise testing could assist in the diagnosis of asthma severity in children [36]. The observation that sodium cromoglycate could markedly reduce severity of EIA led to the suggestion that EIA was due to the release of mediators during exercise [37].

During the late 1970s the role of heat and water loss was appreciated as being important stimuli for EIA [38–42], and protocols for exercise testing have been modified to take this into ac-

count [22, 43]. Exercise *per se* was not essential to cause airway narrowing, and eucapnic hyperpnea could induce similar changes in airway resistance [40, 44]. This led to the development of the eucapnic voluntary hyperpnea (EVH) test as a surrogate test to identify EIA [45]. The EVH test was standardised by members of the US army and used to assess recruits for EIA, making testing more rapid to perform, and less expensive in terms of equipment and human resources than exercise [29, 46]. Eucapnic voluntary hyperpnea was recently recommended by a panel of the IOC Medical Committee (IOC: International Olympic Committee) as the optimal laboratory challenge to identify EIA [47].

Bronchial provocation tests using osmotic aerosols

The development of BPTs using aerosols of hypertonic saline came about as a consequence of the investigations to determine if dry air hyperpnea caused airway narrowing by increasing the osmolarity of the airway surface. The original study of Schoeffel et al. [48] demonstrated that inhaling aerosols of hypertonic saline and hypertonic dextrose caused airway narrowing in asthmatics with EIA, but not in healthy subjects. The mechanism by which the airways narrow in response to an increased osmolarity was suggested to be the release of histamine from mast cells lying superficially in the bronchial mucosa [48]. At the time there was *in vitro* evidence to support this as basophils from asthmatics released histamine when challenged with hypertonic mannitol [49].

The potential to use hypertonic saline as a BPT was recognised immediately. The hypertonic saline challenge was developed initially using 3.6% saline [50] and subsequently 4.5% saline [51]. A good relationship was found between the sensitivity of an asthmatic to hyperpnea and to 4.5% saline and exercise [52]. The advantage of saline challenge was that the airway narrowing developed during the challenge making it safer than challenge by exercise or voluntary hyperpnea where the bronchoconstriction occurred after the challenge had ceased [53, 54].

The importance of airway inflammation in determining the sensitivity to osmotic aerosols was recognised when it was shown that regular treatment with inhaled corticosteroids could reduce or even abolish responsiveness to hypertonic saline [16, 17, 55]. Thus it was proposed that hypertonic saline might be a useful challenge to evaluate the effects of inhaled corticosteroids [55].

The airway sensitivity to hypertonic saline is reduced and often completely inhibited using nedocromil sodium [56] and sodium cromoglycate [17, 50]. Nedocromil sodium and sodium cromoglycate are both thought to protect against hypertonic saline by preventing the release of inflammatory mediators from mast cells and sensory nerves [17, 56], and this is supported by *in vitro* findings [57, 58]. It has been proposed that this inhibitory action on mediator release may relate to the effect these drugs have on cell volume regulation under conditions of osmotic stress [59]. This effect is possibly related to their effect on ion channels [60].

Hypertonic saline challenge is now used in epidemiological settings to assess prevalence of asthma [31, 32, 61]. Hypertonic saline has been shown to be useful as a screening tool to identify "at risk" persons who have a past history of asthma and who wish to dive with self-contained breathing apparatus [62]. Hypertonic saline has also been used in an occupational setting to identify AHR [32]. It is also used in combination with sputum induction so that a measure of inflammatory cell number is made at the same time as airway responsiveness [63, 64].

Inhaled mannitol – a new indirect test for airway hyperresponsiveness

Although "indirect" BPTs have the advantage of being specific for identifying currently active asthma, most are time-consuming to perform and require expensive equipment that is bulky and suitable only for a laboratory setting. In an effort to make "indirect" BPTs faster, portable and needing fewer resources, Anderson et al. have developed a dry powder of mannitol suitable for inhalation [65]. The mannitol powder is encapsulated and delivered in progressively increasing doses using a simple commercially available dry powder inhaler such as the Inhalator™ (Boehringer Ingelheim Pty Ltd, Ingelheim, Germany) [65].

The test is a cumulative dose challenge that is performed by asking the patient to inhale increasing doses consisting of 0 (empty capsule acting as a placebo), 5, 10, 20, 40, 80, 160, 160 and 160 mg of mannitol. The doses of 80 mg and 160 mg are administered in multiple doses of 40 mg capsules, and the total number of capsules containing mannitol required for the challenge test is eighteen. After inhalation of each capsule patients are instructed to perform a 5-second breath hold, then 60 sec after the complete dose is given at least two repeatable FEV_1 manoeuvres are performed and the highest FEV_1 is recorded. The FEV_1 value

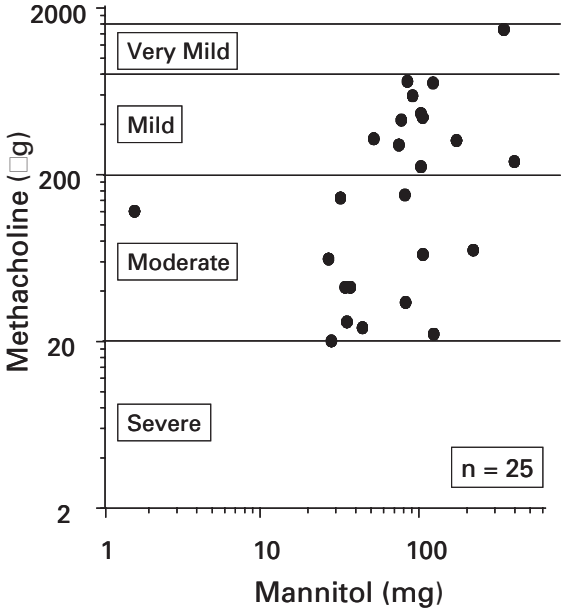

Figure 1

The association between the PD_{15} of mannitol and the PD_{20} of methacholine for individual asthmatic subjects. The data are redrawn from those published by Anderson et al. [65]. The original data were expressed in micromoles and the equivalent values are now expressed in micrograms (□g). The values for mild, moderate, and severe are those commonly used to describe bronchial responsiveness to methacholine.

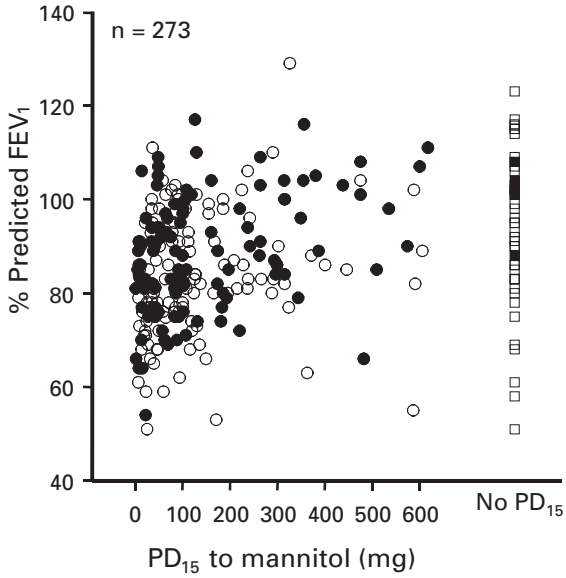

Figure 2

Forced expiratory volume in one second (FEV_1) as a percentage of the predicted value [2] in relation to the provoking dose of mannitol to cause a 15% reduction in FEV_1 (PD_{15}) for 273 asthmatic subjects who performed a challenge with mannitol. Those responsive (i.e. have a PD_{15}) who were not taking ICS are illustrated as the closed circles (rp = 0.35, n = 112), those responsive and taking ICS as the open circles (rp = 0.27, n = 117). Those 44 subjects who were not responsive to mannitol (i.e., No PD_{15}) and taking ICS are illustrated as open squares, and those not responsive and not taking ICS as closed squares.

measured after the 0 mg capsule is taken as the pre-challenge FEV_1 and is used to calculate the percentage decrease in FEV_1 in response to the mannitol challenge. If the patient has a greater than 10% fall in FEV_1 in response to a single dose, the same dose is repeated. The challenge is completed when a 15% fall in FEV_1 is documented or a cumulative dose of 635 mg has been administered. A standard dose of $beta_2$ agonist is administered following the challenge to assist in returning lung function to baseline values.

Mannitol is effective at identifying asthmatic subjects who are also responsive to hypertonic saline, eucapnic hyperpnea, and exercise [65, 66]. As with adults [65] (figure 1) children with current asthma who are also responsive to methacholine [67] are identified using mannitol. Mannitol challenge demonstrates good repeatability, and this has been observed in both adults [65, 68] and children [67, 69].

Non-asthmatic healthy adults and children with no current or past history of asthma or an immediate family history of asthma or other lung disease with normal spirometry do not have any significant reduction in FEV_1 following a mannitol challenge [65, 67]. After a mannitol challenge of a cumulative dose of 635 mg, for the adults the mean (SD) reduction in FEV_1 was 1.7 ± 1.9% (Range: 0–6.2%) (n = 23) [65, 70] and for the children the mean (SD) reduction in FEV_1 was 3.4 ± 2.9% (Range: 0–7.9%) (n = 10) [67].

A retrospective analysis of the mannitol challenge tests performed in 275 subjects (aged 13–70 yr, mean 29 yr median 26 yr) in our laboratory, including data from published studies [18, 65–68, 71, 72], highlights some of the practical features of the mannitol challenge. The median time to complete a mannitol BPT in the 84% (229/273) of asthmatics responsive to mannitol was 12 minutes (Range: 3–27), and the median number of capsules administered was 6 (Range: 1–18). Of all subjects responsive to mannitol the Gmean PD_{15} for this group was 116 mg (95%CI: 99, 135). There were 44/273 (16%) asthmatics unresponsive to mannitol, and the median time to administer the entire eighteen capsules was 20 min (Range:16–30). The pre-challenge FEV_1, expressed as a percentage of the predicted value [2], in relation to the sensitivity to mannitol, measured by the PD_{15}, is given in figure 2. The majority of subjects who were responsive to mannitol had an FEV_1 above 80% of predicted, a value that may be considered within the normal range. Importantly this figure illustrates that asthmatics with good lung function, both treated and untreated, can remain hyperresponsive and spirometry alone is not a guide to existence or severity of AHR to mannitol. Fifty-one percent of those responsive were taking inhaled steroids while 86% of those unresponsive to 635 mg of mannitol were taking inhaled corticosteroids regularly. The only unwanted side effect was excessive cough during challenge in two subjects, and they were not able to complete the challenge.

These tests were performed without significant unwanted bronchoconstriction. The progressive nature of the protocol to administer mannitol means that there is better control over the reduction of lung function compared with other challenges, particularly exercise and eucapnic voluntary hyperpnea. For 86% of 43 patients the reduction in oxygen saturation during the challenge was less than 2%, suggesting that the site of deposition of the mannitol was more likely in the larger airways. For the remaining subjects the fall in saturation was 3% [65]. Recovery to baseline lung function occurred spontaneously, although it was more rapid when a standard dose of $beta_2$ agonist was administered. The time of recovery of the FEV_1 to baseline was similar in adults and children. The actual time for spontaneous recovery appears to depend on the magnitude of the reduction in FEV_1 [65, 68].

At present there have been no population stud-

ies using mannitol, but these are planned for the near future. Mannitol enhances mucociliary clearance [73], and there have been several studies investigating responsiveness in patients with cystic fibrosis and bronchiectasis [74, 75]. In these two groups those subjects, clinically recognised as having asthma, have responded positively to mannitol while those without an asthma diagnosis have not. There has not yet been any systematic study of people with COPD due to smoking, and studies are currently being carried out.

Mechanism of airway narrowing to inhaled mannitol

Mannitol is likely to cause airway narrowing by increasing the osmolarity of the airway surface, an event that causes the release of bronchoconstricting mediators from inflammatory cells in the airways and possibly the sensory nerves [76]. This conclusion has been reached in a number of studies demonstrating directly and indirectly the involvement of inflammatory cells and their mediators in the airway response to mannitol. First, inhaled corticosteroids have been shown to decrease responsiveness to inhaled mannitol in all subjects tested, and this effect was independent of improvements in FEV_1 [77]. As treatment with inhaled corticosteroids reduces inflammatory cell number, particularly mast cells [78] and eosinophils [64], then the response to mannitol is likely dependent upon the presence of these cells, their number and their mediators. Second, nedocromil sodium, which is known to inhibit the release of mast cell mediators and reduce sensory nerve activation, is effective in inhibiting the airway response to mannitol and in 50% of the subjects the response was completely inhibited 15 minutes after nedocromil [71]. It is of interest that with both budesonide and nedocromil the response to treatment was dependent upon the sensitivity (PD_{15}) to mannitol at baseline. Those who were less sensitive (e.g. PD_{15} >200 mg) were more likely to become unresponsive after acute treatment with nedocromil or chronic treatment with budesonide. This is in keeping with the concept that those with milder sensitivity and fewer inflammatory cells will be afforded better protection by drugs that have the ability to target cells that are specific to both this stimulus and the pathology in asthma. Third, the histamine receptor antagonist, fexofenadine, is effective in reducing the airway sensitivity (PD_{15}) to mannitol [68]. Fourth, pretreatment with the cysteinyl leukotriene receptor antagonist, montelukast, resulted in a rapid recovery in lung function while having no effect on airway sensitivity to mannitol [68]. Taken together these findings suggest that mast cells are likely to be involved in the airway response to mannitol. Thus histamine, a preformed mediator, contributes to the immediate airway response to mannitol while newly synthesised mediators such as the leukotrienes sustain the airway response to mannitol. The concept of mast cell involvement is supported by recent findings that there is an increase in levels of a mast cell-specific metabolite of prostaglandin D_2 (9□, 11□-PGF_2) in the urine of asthmatics after a challenge with inhaled mannitol [72]. Leukotriene E_4 levels were also significantly increased in the urine in response to mannitol in the same subjects [72]. Mannitol insufflation in the nose of subjects with allergic rhinitis results in "allergic" symptoms and release of 15-hydroxyeicosatetraenoic acid presumably of epithelial cell origin, but no evidence was obtained to support degranulation of mast cells [79].

Monitoring of therapy in asthma with inhaled mannitol

As one of the primary outcomes of treatment with inhaled corticosteroids is a decrease in the inflammatory cell number, it has been suggested that "indirect" BPTs may better reflect the inflammatory status of the airway following treatment [80, 81]. We have observed that treatment with the inhaled budesonide for 6–9 weeks results in a significant decrease in airway sensitivity to inhaled mannitol in all asthmatic patients (figure 3) [77]. This decrease in AHR was associated with a significant reduction in symptoms and $beta_2$ agonist use in all subjects. Further, responsiveness to mannitol appears to be useful for determining adequacy in dosing with inhaled steroids. While 60% of our subjects remained responsive to mannitol, 40% did not, suggesting that for those who became unresponsive, the dose of steroid was sufficient to reduce the inflammatory cell number and concentration of mediators to less than that required to cause airway narrowing under provoked conditions.

In a recent study, responsiveness to inhaled mannitol as measured by the response dose ratio had 70% sensitivity for predicting failure to reduce inhaled steroids successfully [18]. In the same study sputum eosinophilia was also shown to be a predictor of failed reduction in dose of steroids. Airway hyperresponsiveness to both "direct" (his-

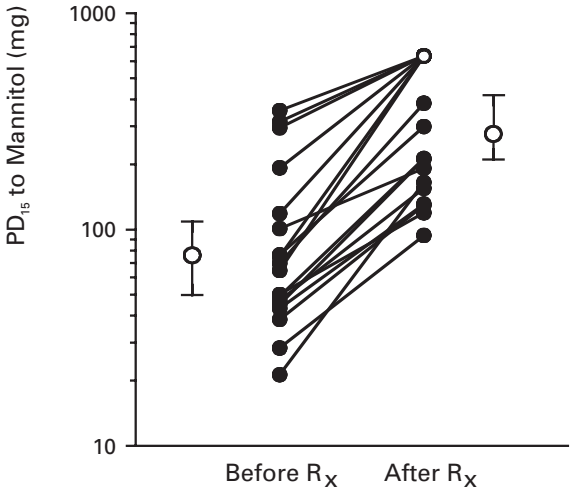

Figure 3

Values for individual data and geometric mean (95% CI) showing an increase in the dose of mannitol to provoke a 15% fall in FEV_1 (PD_{15}) following 6–9 weeks treatment with budesonide compared to before treatment in 18 asthmatic subjects. Following budesonide treatment, seven of the 18 subjects no longer responded to mannitol and have simply been assigned a PD_{15} of 635 mg designated by the open circle. Eleven subjects still recorded a 15% fall in FEV_1 and thus remained positive to mannitol following budesonide. However, the dose of mannitol required to cause the same 15% fall in FEV_1 was significantly greater. Taken from Brannan et al. [77].

tamine) and "indirect" (mannitol) challenge test at the commencement of the dose-reduction phase [82] was a clear predictor for failure of inhaled corticosteroid reduction [77]. The subjects studied were clinically well controlled and symptom-free before the failed reduction of inhaled corticosteroids, suggesting that mannitol responsiveness and sputum eosinophils provide information additional to that provided by symptoms alone. Further studies are being conducted to establish the use of mannitol for monitoring anti-inflammatory therapy in asthma.

Conclusion

Although the utility of pharmacological agents is recognised for identifying hyperresponsiveness of bronchial smooth muscle, these agents would not appear to be as useful as the physical stimuli for a specific diagnosis of asthma or for monitoring therapy. The reason for this probably relates to the nature whereby these different agents act to cause the airways to narrow. Histamine and methacholine are pharmacological agonists and act via specific receptors to cause bronchial smooth muscle contraction, and as a consequence of this the airways narrow. Thus the airway response is not dependent on the presence of inflammatory cells and their mediators. For this reason airway hyperresponsiveness can still be documented after years of treatment with steroids and in the absence of active airway inflammation. By contrast, a positive response to the physical challenges (that alter airway osmolarity) depends on the presence of inflammatory cells and the release of endogenous mediators, probably in response to changes in cell volume from osmotic stress. Thus a positive response to a physical challenge is indicative that inflammatory cells are present in sufficient numbers to have a significant concentration of mediators to which the bronchial smooth muscle is responsive. Failure to have a positive response to mannitol after treatment with inhaled steroids is likely to indicate that the inflammatory cells and their mediators are no longer present in sufficient numbers or concentration to cause the bronchial smooth muscle to contract. The airway response to all the physical stimuli is inhibited by the acute administration of sodium cromoglycate, nedocromil sodium and leukotriene antagonists. Long-term treatment with inhaled steroids markedly reduces responsiveness to physical stimuli and may result in the subject becoming unresponsive. Both "direct" and "indirect" challenge tests give us complementary information, and being hyperresponsive to both has been shown to be a very good predictor for failure of dose reduction of steroids. However "indirect" challenge tests, and mannitol in particular, have been shown to be useful not only in identifying those who will respond to treatment with anti-inflammatory agents but also in guiding reduction in steroid dose. Should this test become commercially available it is likely to be helpful in the identification and management of airway hyperresponsiveness resulting from airway inflammation.

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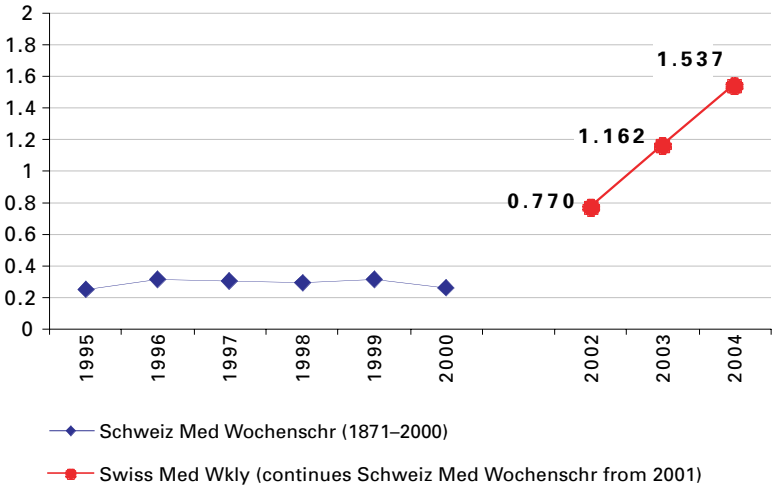

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