Mechanisms of action of bronchial provocation testing

TSANZ / ANZSRS Masterclass: April 3rd, 2016
13:00 – 13:30

John D. Brannan PhD
Scientific Director - Dept. Respiratory & Sleep Medicine
John Hunter Hospital, New Lambton NSW
john.brannan@health.nsw.gov.au
Conflict of interest

• The patent for inhaled mannitol for bronchial provocation is owned by my prior employer Sydney South Western Area Health Service (SSWAHS) and they receive royalties from the sale of Aridol™/Osmohale™ Pharmaxis Ltd (Australia).
  - I receive 10% of the royalties paid to the SSWAHS.

• I own shares I purchased myself in Pharmaxis Ltd.
Outline

- Mechanisms: Overview

- Evidence: Direct BPTs (e.g., methacholine)

- Evidence: Indirect BPTs (e.g., exercise, mannitol)
Definition of Bronchial or Airway Hyperresponsiveness

- Airway hyperresponsiveness is characterised by airways narrowing **too much and too easily** in response to a **wide variety of provoking stimuli**.

- These stimuli include aeroallergens, exercise, cold air, sulphur dioxide, aerosols of distilled water, hyperosmolar agents and specific receptor agonists.
Laboratory Exercise

Exercise in the field

Test for Airway Hyperresponsiveness (AHR)

4.5% saline

Adenosine

Methacholine or Histamine

dry powder mannitol

Eucapnic voluntary hyperpnea
Allergen Challenge

Allergen Inhalation

Mucosal presentation of allergen

Allergen-IgE complex

Epithelium

Submucosa

Presence of increased cellular inflammation
e.g., mast cells (Fc epsilon R1, A2B receptors), eosinophils

Mediator Release from cellular inflammation

Sensitive bronchial smooth muscle

Bronchial smooth muscle contraction & airway narrowing

Augmented during late airway response to allergen

Respiratory Water Loss

Mucosal Dehydration

Increase in osmolarity of airway surface liquid

Exercise

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Natural Stimuli

Surrogate Stimuli

Exercise

Allergen Challenge

Eucapnic Voluntary Hypnoea

Hypertonic aerosols e.g., hypertonic saline, mannitol

Adenosine Monophosphate (AMP)
Respiratory Water Loss

Mucosal Dehydration

Increase in osmolarity of airway surface liquid

Epithelium

Presence of increased cellular inflammation e.g., mast cells (Fc epsilon R1, A2B receptors), eosinophils

Submucosa

Mediator Release from cellular inflammation

Sensitive bronchial smooth muscle

Bronchial smooth muscle contraction & airway narrowing

Mast cells are present in airway epithelium of healthy & asthmatic subjects

Healthy: 8,053 median (IQR 1996 - 24,420) per cubic mm
Asthmatic: 15,371 median (IQR 9,533 - 25,701) per cubic mm

Mast cells & eosinophils as source of mediators that cause bronchial smooth muscle to contract

**Indirect:** the stimulus comes from cells e.g., the mast cell, eosinophil

**Direct:** methacholine acts directly on the receptors on smooth muscle
Human Lung Mast cells release histamine in response to osmotic stimulus


- Mannitol
- Mannitol + Anti-IgE

% Histamine Released

Osmolarity (mOsm / kg H2 O)
Mast cell activation: Effects of Pharmacotherapy

Allergen

- Increase in osmolarity
- Histamine
- PGD<sub>2</sub>
- LTC<sub>4</sub>

Histamine antagonists & Beta<sub>2</sub> agonists protect

Bronchial smooth muscle

Regular use of ICS decreases mast cell (and eosinophil) number and thus source of mediator.

Beta<sub>2</sub> agonists inhibit release of mediators.

Sodium cromoglycate prevents release of PGD<sub>2</sub>.

LT antagonists
‘Direct’ bronchial provocation tests

**DIRECT challenge test:** e.g. methacholine, histamine

The agent is administered and acts on a specific receptor on the bronchial smooth muscle causing it to contract and the airways to narrow. Identifies bronchial responsiveness consistent with asthma or with airway injury / airway remodeling.

*Identifies airway smooth muscle sensitivity to the administered substance*
Eosinophils (%) vs airway sensitivity to methacholine (PD$_{20}$)

$$r_p = -0.28$$
$$p = \text{ns}$$
$$n = 38$$

Change in direct BHR to inhaled corticosteroids

Difficult to abolish with regular ICS

PD_{20} μg

PC_{20} mg/ml

Reddel 1600 64
Du Toit 1000 12
Jenkins 500 6
Foresi 1000 6
Lim 1600 4
Sont 800 104

Brannan et. al. Clin Respir J 2007; 1
‘Variable’ vs ‘Fixed’ component of direct AHR

“…..25 or 30 years ago a concept developed indicating that (direct) AHR had two components: variable and fixed. The variable component changes sometimes rapidly (e.g., with allergen exposure, ICS), and the fixed component remains despite, at times, intensive treatment of asthma.
‘Variable’ vs ‘Fixed’ component of direct AHR

“…..25 or 30 years ago a concept developed indicating that (direct) AHR had two components: variable and fixed. The variable component changes sometimes rapidly (e.g., with allergen exposure, ICS), and the fixed component remains despite, at times, intensive treatment of asthma.

- variable component reflects changes in airway inflammation
- fixed component reflects structural / functional changes in the airway that are persistent, if not permanent, and that have been labelled ‘airway remodelling’.

AHR in elite skiers: airway injury

Airway Responsiveness to histamine in children with and without EIB

<table>
<thead>
<tr>
<th>EXERCISE</th>
<th>HISTAMINE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall in FEV$_1$ &gt;15%</td>
<td>n 27</td>
<td>+ve 77%</td>
</tr>
<tr>
<td>Fall in FEV$_1$ 10-14.9%</td>
<td>50</td>
<td>22%</td>
</tr>
<tr>
<td>Fall in FEV$_1$ &lt;10%</td>
<td>387</td>
<td>12%</td>
</tr>
</tbody>
</table>

| Fall in FEV1 >15% | 40 | 55% | 45% |
| Fall in FEV1 <10% | 161 | 12% | 88% |
| Haby et al. 1995 Eur Respir J 8:729-36 & MSc U Syd |
EIB in adults and AHR to methacholine

Methacholine in subjects with unclear diagnosis of asthma:

- A high rate of methacholine negative test results (49%) in those given a clinical diagnosis of asthma by a respiratory physician at the end of the study.

- The importance of this observations is that it is subjects with these characteristics who are most likely to be referred for testing.

Anderson SD et al., Resp Res 2009; 10:4
Airway responses to agonists

% Change in FEV₁ vs Concentration (M)

- LTD₄
- PGD₂
- PGF₂α
- Methacholine
- Histamine

Concentration (M):
- 10⁻⁵
- 10⁻⁴
- 10⁻³
- 10⁻²
- 10⁻¹

O'Byrne Chest 1997; 111:27S-34S
‘Indirect’ bronchial provocation tests

INDIRECT challenge test: e.g. mannitol

“Indirect challenges act by causing the release of endogenous mediators that cause the airway smooth muscle to contract, with or without effect in inducing microvascular leakage”.


Identifies the presence and interaction of the two key pathophysiological features of asthma
Grades apply to those not taking inhaled steroids
Intraepithelial Tryptase+/CPA3+ mast cells in EIB

Lai et al. *JACI* 2014: 133:1448
Urinary $9\alpha 11\beta$-PGF$_2$ release before and following exercise

$9\alpha, 11\beta$-PGF$_2$ ng.mmol creatinine$^{-1}$

Before | 30 min | 90 min
---|---|---
EIB+ (n=7) | | |
EIB- (n=5) | | |

In those with EIB while sputum eosinophilia is common the % of eosinophils can be within the normal range of <2%

Spearman's rho = 0.6 (p < 0.01)

24% of 278 adults and 95 children had a positive exercise test on one occasion and negative on another.

Exercise 1 to predict Exercise 2: 62% sensitivity

Anderson SD et al., Resp Res 2010; 11:120
Dose response to inhaled mannitol

% Fall FEV1 vs Cumulative dose of mannitol (mg)

- Severe: ≤ 35mg
- Moderate: ≤ 155mg
- Mild: > 155mg

RDR = Response (%) / Final/Max Dose (mg)

PD$_{15}$: 'airway sensitivity'

'airway reactivity'

Normal
Relationship between the % Fall in FEV$_1$ after exercise compared to the airway sensitivity to inhaled mannitol (PD$_{15}$) in steroid naïve asthmatics

Brannan AJRCCM 1998; 158:1120-6

Munoz J Appl Physiol 2008; 105:1474
Eosinophils (%) vs airway sensitivity to mannitol (PD$_{15}$) mg

$r_p = -0.52$
$p < 0.05$
$n = 38$

Relationship between mast cell number and responsiveness to 4.5% saline in corticosteroid treated asthmatic adults

Gibson et al, 2000 JACI 105:752
Serum IgE in relation to airway sensitivity to mannitol in ICS naive asthmatics

$r_p = -0.69, p = 0.0004, n = 22$

Baraket PhD Thesis 2007, University of Sydney
Effect of therapies on the airway response to mannitol

- Budesonide 1156 μg/day
- Nedocromil 8 mg
- Fexofenadine 180 mg

AAC % Change min:
- Montelukast 10 mg
Prostaglandin $D_2$ release blocked by SCG and eformoterol

Brannan et al. Eur Respir J 2006; 27: 944-950
Airway response to mannitol after 6-9 weeks of budesonide (800-2400mcg)

- BEFORE $R_X$: 78 mg (51, 117) mg
- AFTER $R_X$: 289 mg (202, 414) mg

50% No PD15

Response Dose Ratio to Mannitol

RDR = Response (%) / Dose (mg)

Brannan Respirology 2002; 7:37-44
Differences in airway reactivity (RDR) between asthmatics taking ICS and non-asthmatics

<table>
<thead>
<tr>
<th>Response Dose Ratio (mg/%Fall)</th>
<th>GeoMean (95%CI)</th>
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</thead>
<tbody>
<tr>
<td>Asthmatic taking ICS</td>
<td></td>
</tr>
<tr>
<td>p&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>n=129</td>
<td>91±12%</td>
</tr>
<tr>
<td>Median PD$_{15}$ 182mg</td>
<td></td>
</tr>
<tr>
<td>n=123</td>
<td>99±15%*</td>
</tr>
<tr>
<td>n=80</td>
<td>107±12%</td>
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</tbody>
</table>

Data from: Brannan et. al. Respiratory Research 2005; 6: 144

Conclusion

- **Indirect:** An airway response to dry air hyperpnea and osmotic stimuli require the presence of both *airway inflammation* (e.g., mast cells, eosinophils) and a *responsive airway smooth muscle.*

- Highly suggestive of a benefit to treatment with ICS

- **Direct:** Airway responses to direct stimuli (e.g., methacholine) comprises of a modifiable and a fixed component (?airway injury).

- AHR to methacholine may not be present in EIB alone or in association with newly presenting clinical signs and symptoms of asthma.