Monitoring mast cell activation by prostaglandin D₂ in vivo

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Prostaglandin D₂ is a useful in vivo marker of mast cell activation in humans

While the pro-inflammatory role of eosinophilic granulocytes in asthma is currently under debate, an increasing body of evidence suggests that mast cells may indeed orchestrate many of the characteristic pathophysiological changes in asthma. There are also indications that the mast cell may be an effector cell in other lung diseases such as chronic obstructive pulmonary disease and lung fibrosis. Given the location of mast cells at multiple sites within the airways, they clearly have the potential to function as sensors of alterations in the microenvironment—be it to inhaled or bloodborne substances, microbes, or other insults that require a prompt host defense reaction. Their versatility is demonstrated by the great number of stimuli that trigger mast cell activation (fig 1). In addition to classical IgE-dependent degranulation of mast cells, transduction pathways resulting in mast cell activation may be triggered by, for example, adenosine, hyperosmolarity, and lipopolysaccharide.

**MAST CELL MARKERS**

Although many mast cell mediators or products serve as useful markers of mast cell activation in vitro, it has been notoriously difficult conclusively to establish mast cell activation in human studies. For example, it is difficult to catch the short lived increase in plasma levels of histamine and its metabolites following allergen induced bronchoconstriction. Furthermore, circulating basophils may contribute significantly to plasma histamine and plasma values may be increased by non-specific challenges such as an ordinary exercise test. Measurements of urinary metabolites of histamine may sometimes be helpful as specific markers for any particular cell. However, in this issue of *Thorax*, Bochenek et al confirm and extend the accumulated evidence that measurement of PGD₂ or its metabolites represents a sensitive and reliable strategy for assessment of mast cell activation in vivo. Specifically, they convincingly show, for the first time, increased levels of the primary PGD₂ metabolite 9α,11β-PGF₂α in plasma during the early phase of allergen induced airway obstruction. This is achieved by applying gas chromatography-negative ion chemical ionisation-mass spectrometry (GC-NICI-MS) to samples collected at frequent intervals before and during allergen bronchoprovocation of subjects with atopic asthma. The methodolgy is very appropriate as GC-NICI-MS is the most specific measurement of this particular family of compounds, where the presence of numerous structurally related metabolites almost complicates immunoassay measurements. Bochenek et al also deserve credit for their development of a protocol that improves the sensitivity of the GC-NICI-MS measurements.

**BIOSYNTHESIS OF PGD₂ IN MAST CELLS**

The release of PGD₂ from isolated human mast cells was reported more than two decades ago, shortly followed by the demonstration of its release into human airways after local endotracheal instillation of allergen. However, the mechanistic significance of these reports was not generally appreciated. In humans, mast cells are an almost exclusive cellular source of PGD₂. Although there is evidence of some PGD₂ formation by platelets, macrophages and certain T lymphocytes, the reported amounts are 100–1000 times lower than those produced during IgE-dependent activation of mast cells. More importantly, whereas the basophil and the mast cell both release histamine and leukotriene (LT) C₄, it is only the mast cell that produces significant quantities of PGD₂. There is, in fact, recent evidence to show that increased expression of the haemato poetic PGD₂ synthase may be the functional response that is most specifically upregulated in activated mast cells.

**MEASUREMENT OF PGD₂**

The currently renewed interest in applications of PGD₂ measurement would not have been possible without the comprehensive work of Roberts and colleagues at Vanderbilt who performed painstaking GC/MS identifications of PGD₂ metabolites in blood and urine after injections of radiolabelled PGD₂. More than 25 metabolites were identified but intact PGD₂ was not found in the urine. The most abundant PGD₂ metabolite identified was 9,11-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20 dioc acid, commonly referred to as PGD-M. The earliest appearing urinary metabolite was 9α,11β-PGF₂α, which was subsequently shown to be stereospecifically transformed from PGD₂ by the NADPH dependent enzyme 11-koereductase in lung and liver. Interestingly, 9α,11β-PGF₂ retains biological activity. It has, for example, been found to contract bronchial smooth muscle and has vascular effects including contraction of coronary arteries. Metabolism of 9α,11β-PGF₂ by the 15-hydroxy prostaglandin dehydrogenase, followed by β- and ω- oxidations, leads to PGD-M.

The Vanderbilt group thus used GC/MS measurements of PGD-M as a marker of systemic PGD₂ production in different disease states. Markedly raised levels of PGD-M were discovered in systemic mastocytosis as well as during anaphylaxis. The GC/MS approach is, however, laborious and technologically demanding, which generally renders it less applicable to studies of populations and large numbers of samples. The more recent validation of an immunoassay method for the measurement of 9α,11β-PGF₂ in urine has therefore created new opportunities for
Figure 1 Mast cells may produce a large number of mediators, enzymes, cytokines and other factors in response to allergic (IgE dependent) or non-allergic activation (adenosine, exercise, endotoxin, mannitol, non-steroidal anti-inflammatory drugs (NSAIDs) in NSAID intolerant subjects, etc). However, only tryptase and prostaglandin (PG) D₂ (boxed) are specific markers of mast cell activation. As reported by Bochenek et al in this issue, measurement of PGD₂ and its metabolites is currently the most sensitive strategy to monitor mast cell activation in human subjects.

using this PGD₂ metabolite as a mast cell marker. Using this immunoassay methodology, increased excretion of metabolites of PGD₂ into the urine has been observed after allergen induced bronchoconstriction and mast cell involvement in other indirect challenges has also been confirmed.

As discussed by Bochenek et al., apart from weak indirect or anecdotal evidence, there has not previously been any investigation of 9α,11β-PGF₂ levels in plasma during allergen induced bronchoconstriction, which undoubtedly must be the gold standard for mast cell activation. Interestingly, the current demonstration of increased PGD₂ release during allergen induced bronchoconstriction puts further weight behind a previous publication from the group in Krakow where increased plasma levels of 9α,11β-PGF₂ were found following aspirin induced bronchoconstriction. This adds to several other lines of evidence suggesting that the intolerance to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) involves mast cell activation.

Bochenek et al confirmed the original observations by Liston et al. that 9α,11β-PGF₂ was the first PGD₂ metabolite to appear in urine, although they did not find the increase of this metabolite in the urine to be as great as that reported by O’Sullivan et al. These seemingly different findings are most probably explained by the demonstration that the immunoassay measures not only 9α,11β-PGF₂ but also at least two other metabolites that appear somewhat later. In other words, for detection of the sum of the initially excreted PGD₂ metabolites in urine, the chemically less specific immunoassay will paradoxically have greater practical sensitivity as it measures several related PGD₂ metabolites. However, as pointed out by Bochenek et al, for studies of the kinetics of 9α,11β-PGF₂ metabolism, the chemically more specific method is obviously preferable.

PERSPECTIVES

The method chosen to monitor mast cell activation by measurement of PGD₂ metabolites will obviously depend on the questions asked and the resources available. Irrespective of the analytical method selected, measurements of 9α,11β-PGF₂ in plasma, urine, or other body fluids currently provide the most sensitive method for detection of mast cell activation in vivo. This was clearly shown in the paper by Bochenek et al, where there was no change in plasma tryptase despite the fivefold increase in plasma 9α,11β-PGF₂. Similarly, in previous work by O’Sullivan et al, there was consistently a much smaller or non-significant increase in urinary methyl histamine in contrast to consistent and prominent increases in urinary 9α,11β-PGF₂ metabolites. Thus, for investigations into the role of the mast cell in different pulmonary diseases, measurements of PGD₂ metabolites in body fluids offer many new opportunities.

Finally, PGD₂ is not only a marker of mast cell activation but also—together with its immediate metabolite 9α,11β-PGF₂—it is a potent mediator of bronchoconstriction, vasomotor tone, and cell recruitment. We hypothesise that PGD₂ mediates the component of allergen-induced bronchoconstriction that remains resistant to antihistamines and antileukotrienes. Experimental data are available to support such a role, and a role for PGD₂ in rhinitic responses in humans has also been implicated. The recent awareness that there are at least three different receptors (TP, DP, and CRTH2) mediating the effects of PGD₂ in the airways suggests that we may soon get improved opportunities to define more precisely the pulmonary role of this mast cell derived mediator.


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Breathlessness during exercise in COPD

P M A Calverley

Salmeterol reduces breathlessness during exercise without necessarily changing exercise duration

The inability to exercise because of distressing breathlessness is one of the most frequent problems experienced by patients with chronic obstructive pulmonary disease (COPD) and is a major determinant of impaired quality of life. Our understanding of why this occurs and how best to treat it has improved significantly in the last decade. At one level the problem appears relatively straightforward. Exercise invariably involves an increase in whole body oxygen consumption and carbon dioxide production, which requires an appropriate rise in alveolar ventilation if arterial carbon dioxide tension is to remain constant. In patients with COPD the capacity to increase minute ventilation is restricted as is the capacity to empty their lungs quickly, hence exercise limitation occurs at a lower workload than in age matched healthy subjects. Although there is much truth in this simple scheme, it does not do justice to the many complex adaptive responses that patients use to cope with their chronic airflow obstruction, nor does it explain the variability seen in both the duration of exercise and the intensity of breathlessness in patients with apparently similar levels of airflow obstruction.

ADAPTATIONS TO REDUCED EXERCISE CAPACITY IN COPD

Unsurprisingly, the general relationship of ventilatory capacity, commonly established indirectly from the forced expiratory volume in 1 second (FEV₁), is not simple. While the 12 minute walking distance is broadly related to the severity of airflow obstruction, spirometric measurements are not very precise indicators of exercise capacity in the individual patient. Differences in ventilation-perfusion matching during exercise mean that individuals with more “wasted ventilation” achieve their maximum sustainable ventilation sooner for the same degree of alveolar ventilation. Differences in pre-morbid fitness are also relevant. Patients who are less fit progress to anaerobic metabolism (and hence increased carbon dioxide production) at lower levels of work than those who are fitter. More recently, differences in the dynamic behaviour of the respiratory system during exercise have been identified. Unlike healthy subjects, patients with severe COPD progressively increase their end expiratory lung volume rather than reducing it during exercise as occurs in healthy subjects. Since total lung capacity is constant, these patients have a restricted ability to increase tidal volume and their minute ventilation is increased predominantly by an increase in respiratory frequency. The ability to eliminate carbon dioxide is even worse in these circumstances and some patients become hypercapnic before stopping exercise. More recently, changes in end expiratory chest wall volume—which reflects changes in lung volume—have been shown to occur during uninstrumented exercise. This pattern of response is not universal as some patients appear to retain the more normal behaviour of trying to reduce end expiratory lung volume while exercising, which perversely may be a bad strategy when they are close to flow limitation at rest. Nonetheless, patients with the most severe COPD, and certainly those with the lower expiratory flow reserve, adopt a strategy of allowing dynamic hyperinflation to occur which further compromises their respiratory mechanics and increases their sensation of breathlessness.

Changes in lung volume with exercise provide an attractive explanation for the...
increase in breathlessness reported by patients with COPD as respiratory muscle activation, a key correlate of breathlessness in patients with a mechanically impaired lung function, is increased at any given workload. The higher the lung volume, the less effective is respiratory muscle contraction due to shortening of the respiratory muscles, particularly the diaphragm. Moreover, breathing occurs over a flatter part of the pressure-volume relationship of the respiratory system and is mechanically less efficient. These processes have been described as neuromechanical dissociation as there is an increase in respiratory drive that fails to produce effective ventilation. By analogy with the previously popular length-tension inappropriate concept of Campbell and Howell, it is believed that neuromechanical dissociation is a major determinant of breathlessness in COPD. Certainly, there is increased respiratory muscle energy expenditure which can approach the levels where inspiratory muscle fatigue can occur. However, whether this actually happens during exercise in patients with COPD remains contentious.

**DRUG TREATMENT: MECHANISMS OF ACTION**

As mechanical factors appear to predominate, it is reasonable to try to alleviate these with drugs that improve lung emptying, principally by producing airway smooth muscle relaxation. Initial data were rather disappointing as they focused on the magnitude of change in FEV1 (modest at best) and whether individuals showed bronchodilator reversibility. This is not a particularly effective approach as the specificity of reversibility testing in stable COPD is poor, as is the relationship of acute changes in these variables to subsequent exercise performance. Studies with β agonists and anticholinergic drugs have shown that there is improvement in self-paced or treadmill exercise tests irrespective of the magnitude of lung function change or the presence of oxygen desaturation. O’Donnell and colleagues reported improvements in end expiratory lung volume during exercise in patients treated with ipratropium, which suggests that the bronchodilator delayed the onset of dynamic hyperinflation. Similar changes in end expiratory lung volumes at comparable workloads have been seen with β agonists, although in this case the duration of exercise did not change.

In this issue of Thorax Man et al provide further insight into this problem. They studied 16 patients selected as having “irreversible” COPD with a mean change in FEV1 after an inhaled bronchodilator of only 10 ml, although it is not clear what dose of bronchodilator was used and how long after the measurement was made. However, treatment with regular salmeterol produced an improvement in FEV1 of only 40 ml, so the patients must be assumed to be relatively unresponsive, at least as judged spirometrically. Patients were randomised into a double blind, placebo controlled, crossover study where the change in transdiaphragmatic pressure-time product and end expiratory lung volume were compared while taking either a placebo or twice daily salmeterol in conventional doses. When compared at the same time point during exercise, the long acting β agonist significantly reduced the transdiaphragmatic pressure-time product, the degree of dynamic hyperinflation, and the severity of breathlessness. However, patients treated with salmeterol did not walk further, even though they were less breathless.

The reduction in respiratory muscle pressure-time product is in keeping with earlier data showing that the resting EMG, an index of respiratory muscle activation, was reduced after an inhaled β agonist. The reduction in transdiaphragmatic pressure-time product was largely the result of a fall in the gastric pressure, emphasising the importance of activation of the abdominal muscles during exercise in COPD. Resting inspiratory capacity fell by approximately 160 ml while the degree of dynamic hyperinflation was about 110 ml less after salmeterol. The similarity in magnitude of these changes suggests that the bronchodilator operates by shifting the starting point from which dynamic hyperinflation begins rather than changing its rate of evolution. The change in the relationship of tidal volume to oesophageal pressure at isotime was a good predictor of the change in breathlessness after treatment, as was the change in end expiratory lung volume. These findings are in keeping with earlier variables known to influence breathlessness at rest in COPD, particularly respiratory timing and tidal volume. It is a pity that more detail of the breathing pattern at the isotime comparison points was not provided.

These new data give further support to the idea that mechanical factors are the major determinants of breathlessness during exercise in COPD, certainly in patients with this severity of disease. The change in inspiratory capacity produced by salmeterol was similar to that produced at rest by nebulised salbutamol, although whether any further improvement in lung function could have been produced by adding in a different anticholinergic bronchodilator is not addressed here. The reduction in activation of the abdominal muscles produced by the bronchodilator is similar to the change seen by unloading the respiratory system mechanically with non-invasive ventilatory support, and such strategies might be synergistic. These improvements were seen after regular use of the long acting bronchodilator and suggest that there is no immediate tachyphylaxis in the effects of this treatment, at least spirometrically. Lack of improvement in exercise duration may reflect the severity of the patients studied or the complexity of the protocol adopted. It does emphasise the need to evaluate both the breathlessness and distance walked, particularly with current treatments which have only modest effects on reducing the mechanical limitations associated with exercise and COPD. Whether similar benefits are seen in individuals who are not flow limited at rest and are therefore less likely to exhibit dynamic hyperinflation remains to be tested, although at least one study suggests that this may not be the case.

**CLINICAL IMPLICATIONS**

These relatively complex physiological investigations do have clinical implications. Current treatment can produce small changes in easily measured indices of lung function such as FEV1, which translate into more important improvements in respiratory muscle energy consumption and perceived breathlessness. The mean changes in Borg scale reported by Man et al represent a change from severe to somewhat severe, which may not appear important to fit people but is certainly noticed by patients with COPD. Why apparently similar individuals fail to obtain these benefits while others report marked improvement remains to be established, as does the consistency of changes in breathlessness. It is encouraging that changes in dynamic lung volume are as predictive as more invasive balloon catheter measurements in determining those who felt less breathless as this may make it easier to study these problems in future using less intrusive methodologies. The findings of Man et al reinforce the need to ask patients how they feel when they have been taking treatment and give us more confidence that their responses are likely to be physiologically meaningful, an approach endorsed recently in the NICE guidelines on COPD management.

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Quality of life measurement in sleep apnoea

Measuring quality of life in patients with sleep apnoea: whose life is it anyway?

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A new self-administered disease specific questionnaire for sleep apnoea

Patients suspected of suffering from sleep apnoea present to primary care physicians with several typical symptoms including habitual snoring (often disruptive to bed partners), waking feeling unrefreshed, daytime sleepiness, or fatigue. The disorder is increasingly being recognised in patients who are hypertensive, obese, or who have a previously unrecognised respiratory failure. Randomised placebo controlled trials have proved that sleep apnoea has an adverse impact on mood, quality of life, functional status, and vigilance, and that treatment with continuous positive airway pressure (CPAP) results in statistically significant improvements.

Although sleep apnoea is strongly suspected to be a risk factor for developing systemic hypertension, and some preliminary evidence suggests that it is associated with an increased risk for cardiovascular and cerebrovascular disease, there is no convincing evidence yet that CPAP reduces these risks. In most patients treatment is therefore primarily aimed at improving their quality of life. Although symptoms and quality of life are often used interchangeably, the former is a subset of the latter and does not capture the complete impact of the disorder.

When patients are treated for sleep apnoea it is important to document whether the treatment is effective. All too often this evaluation is limited to determining whether the apnoea-hypopnoea index has been satisfactorily reduced. A Medline search of the English language literature for randomised controlled trials of adult sleep apnoea syndromes over the past 10 years produced 95 studies, 40% of which did not report any symptom or quality of life outcomes. It is well recognised that the apnoea-hypopnoea index correlates poorly with these outcomes, so by itself the index is not an appropriate measure. If it is accepted that quality of life is what matters most to patients with sleep apnoea, then clinical trials should include it as an important and possibly the primary outcome measure. Quality of life was included as an outcome measure in only 25% of the 95 clinical trials.

When an investigator is planning to conduct a clinical trial or when a practitioner is reviewing a published study, what expectations should they have for the method by which quality of life is measured? In general there are two categories of quality of life measures—generic and disease specific. Generic indices such as the SF-36 have the advantage that they are in common use and have been used in many disease states so it is possible to compare...
impacts of sleep apnoea and its treatment with other disorders. However, the drawback to these indices is that they may fail to capture important aspects of the impact of sleep apnoea on patients and therefore may be insensitive to important improvements experienced with treatment. In addition, generic indices were designed to compare broad aspects of quality of life at a single point in time across disease states (a discriminative property) and were not specifically designed to measure within-subject change following a therapeutic intervention (an evaluative property).4 Quality of life indices, like physiological measures, should be evaluated for their signal to noise properties before being accepted as important measures in clinical trials. The “signal” in a subjective measure like quality of life is the magnitude of the change in score when patients have improved or deteriorated, a property referred to as **validity**. The “noise” in this type of measure is the amount of change recorded in stable patients who have not yet been offered treatment, a property referred to as **reliability**. Most symptom questionnaires are not rigorously evaluated for these two essential properties or for a third property referred to as **responsiveness**. An instrument is considered valid when it has been shown to measure what it is intended to.

Disease specific questionnaires such as the Quebec Sleep Questionnaire (QSQ) for sleep apnoea described by Lacasse et al.2 in this issue of Thorax are developed by a long and arduous process that begins with patients’ opinions about the most frequent and important aspects of the disorder that impact on their lives. This ensures face validity of the instrument and, in the case of the QSQ, the fact that its items are very close to the similarly designed sleep apnoea quality of life index4 adds to its face validity. The QSQ was shown to have construct validation by demonstrating that within-subject changes in its domains correlate, as predicted, with other quality of life (SF-36, Symptom Checklist-90), functional status (Functional Outcomes in Sleep Questionnaire), and symptom based questionnaires (Epworth Sleepiness Scale). So why not simply use these instruments? Why another one? The QSQ was designed as an evaluative instrument, meaning that the authors included items that patients indicated during the construction phase of the questionnaire would be sensitive to change with treatment. It is therefore more likely to detect small but important changes in the lives of patients with sleep apnoea (signal) than the other instruments. In fact, the authors were able to show that it has a stronger signal than the Functional Outcomes in Sleep Questionnaire. Importantly, it has also been shown to have a relatively small amount of noise (high reliability), as evidenced by its high intraclass correlation coefficient in subjects who completed the questionnaire twice before being offered treatment. It therefore has excellent signal to noise ratio properties.

Perhaps the most important property of a quality of life questionnaire used in clinical trials is the ability of its results to be understood or placed in context. It is also the property most overlooked in quality of life or symptom based questionnaires. It answers the question appropriately asked about many results—“So what?” If a study shows that four out of the eight domains of the SF-36 improve significantly (statistically), what is the clinical significance of this? If one domain improves by 25%, is this a large increase? Does this magnitude of change indicate a life changing event or merely a minor improvement in one aspect of a patient’s life? This property of a questionnaire is referred to as **interpretability** and should be grounded in the experience of patients, not in statistics.2 No generic quality of life instrument or symptom based questionnaires have published evidence on interpretability. Only nine of the published sleep apnoea clinical trials (<10%) used a disease specific quality of life instrument and most of these had not described the property of interpretability. It is therefore difficult, if not impossible, to estimate from the published literature the clinical importance and magnitude of the change in patients’ lives when they have been adequately treated for sleep apnoea.

The QSQ, like the Sleep Apnoea Quality of Life Index, provides “consumers” with a minimal important clinical difference. This draws a line in the sand which allows researchers to describe the percentage of patients improved to this extent, and suggests that a statistically significant mean result that is less than this may not be clinically significant. The QSQ has evaluated this property in a relatively small number of patients and obtained rather large numbers for their minimal important clinical difference—much larger than similarly constructed questionnaires for other disease states and for the Sleep Apnoea Quality of Life Index.4 The exact reason for this discrepancy is not clear from the description of their methods or the discussion of their results. It may be related to the small numbers of patients studied or because they chose to use a self-administered format rather than the interviewer administered format of most other questionnaires. Additional research is required to evaluate this property further. Notwithstanding this, the development of the QSQ establishes an important standard for a self-administered sleep apnoea disease specific questionnaire to which researchers and readers of clinical trials in this field should pay attention.

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