bronchodilator compared with a group receiving placebo.⁹ By the end of the training programme, the duration of a constant work rate treadmill task was increased by 79% in patients receiving bronchodilator treatment compared with those receiving placebo.

Finally, in this issue of *Thorax*, Peters *et al* report that 50% oxygen breathing acts synergistically with bronchodilator therapy (ipratropium bromide) to reduce hyperinflation and improve exercise tolerance in patients with COPD.¹⁰ In a crossover study in 16 patients, the mean tolerance of a constant work rate increased by 57% and 31% when inhaling oxygen or receiving bronchodilator, respectively. The combination of oxygen and ipratropium increased exercise tolerance by an average of 87% over the control condition, demonstrating an approximately additive response.

The mathematically inclined among us will note that only four of six possible permutations of these four interventions have so far been reported. The successful combinations of heliox with exercise training and of bronchodilators with heliox are waiting to be achieved. Further, can studies of the simultaneous application of three interventions be far behind? Four studies of this kind could

be planned, the requirement being the necessity to prove the superiority of the exercise tolerance improvements in response to three interventions over that of three groups in which combinations of two of the three interventions are applied. Will we see the day when a study appears in which simultaneous application of all four of these physiological manoeuvres are combined to ameliorate exercise intolerance and are proved to be superior to three groups in which three of the four interventions are given? One can only dream.

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COX-2 in CF

COX-2: a link between airway inflammation and disordered chloride secretion in cystic fibrosis?

A Clayton, A J Knox

Possible role for COX-2 in the pathophysiology of CF

Lung disease in cystic fibrosis (CF) continues to be the major cause of morbidity and mortality, with the mechanisms whereby the biochemical defect causes lung disease receiving considerable attention. CF is caused by mutations in a 230 kB gene located on chromosome 7 which codes for the cystic fibrosis transmembrane conductance regulator (CFTR).¹ CFTR functions in the main as a cAMP regulated chloride channel in epithelial and glandular structures.² It has long been recognised that the abnormal chloride secretion by bronchial epithelial cells in CF predisposes to the development of bronchial damage and inflammation. The mechanism is

under debate, but altered biochemical constituents of airway surface liquid,^{3–5} function of the mucociliary escalator,⁶ and the function of antibacterial defence molecules such as defensins⁶ are all thought to play a part. What is less clear is whether inflammation itself can feed back to further compromise the abnormalities in chloride transport leading to an amplifactory cycle of lung destruction. Studies of nasal potential difference in patients with CF have shown that there is a range in the severity of chloride flux reduction.^{7–8}

Lipid mediators such as prostanoids have an important role in regulating inflammation in most tissues including

the lung. Prostanoids are produced from membrane phospholipids by a three step reaction involving arachidonic acid release via phospholipase A2, conversion of arachidonic acid into PGH₂ by cyclooxygenase (COX), followed by its conversion to terminal prostaglandins such as PGE₂ by specific synthases and isomerases. COX exists in three isoforms:⁹ COX-1 is constitutively expressed;¹⁰ COX-2 is inducible and therefore implicated in many inflammatory and malignant diseases;^{11–12} and a further enzyme, COX-3, has recently been described whose function is less well defined.¹³ Previous studies have shown that the levels of prostanoids such as PGE₂, PGF₂, PGF₁, and thromboxane B₂ are raised in primary cultures of epithelial cells from the CF airway,¹⁴ suggesting that they may play a part in the pathogenesis of CF. Further evidence to suggest that prostanoids have an important pro-inflammatory role in CF is provided by clinical trials which have shown that the broad spectrum COX inhibitor ibuprofen can delay the progression of CF lung disease.¹⁵

Several questions are raised by these findings.

- What is the mechanism of increased prostanoid release and what is their role in regulating CF disease?

- How might increased COX-2 expression occur in CF?
- How does an increase in COX-2 expression contribute to CF pathophysiology?
- What are the implications of these findings?

WHAT IS THE MECHANISM OF INCREASED PROSTANOID RELEASE AND WHAT IS THEIR ROLE IN REGULATING CF DISEASE?

In this issue of *Thorax* Roca-Ferrer and colleagues¹⁶ investigate the expression of COX isoforms in the nasal epithelium of patients with CF. The most striking feature of their studies was that COX-2 protein was strongly expressed in CF but not in non-CF polyps or in normal mucosa. Furthermore, upregulation of COX-2 protein was more marked than COX-2 mRNA, suggesting possible alterations in transcriptional and post-transcriptional regulation. COX-1 protein was also upregulated in CF, but to a lesser degree as it was strongly expressed in normal mucosa and non-CF polyps. COX-1 mRNA was also slightly upregulated. These results suggest that the marked upregulation of COX-2 is likely to be responsible for the increased prostanoid levels found by others in CF.

HOW MIGHT INCREASED COX-2 EXPRESSION OCCUR IN CF?

There is currently no evidence that alterations in CFTR can directly regulate COX-2, whereas COX-2 upregulation is commonly seen in inflammatory micro-environments such as the CF lung. Perhaps a more plausible explanation is that bacterial products and/or pro-inflammatory cytokines in the inflamed CF lung are responsible for inducing COX-2. Bacterial lipopolysaccharide can induce COX-2 in T84 gut epithelial cells,¹⁷ mouse neurons,¹⁸ and macrophages.¹⁹ We and others have shown that IL-1 β , which is increased in CF,²⁰ can induce COX-2 in airway epithelial cells.²¹ While the results reported by Roca-Ferrer are interesting, a key question is whether or not similar changes in COX expression are found in bronchial epithelium.

HOW DOES AN INCREASE IN COX-2 EXPRESSION CONTRIBUTE TO CF PATHOPHYSIOLOGY?

One hypothesis that we have been pursuing in our laboratory is that COX-2 products can regulate CFTR mediated chloride flux across the airway epithelium. In experiments in a Calu-3 bronchial epithelial cell line which expresses

functional CFTR channels in bronchial epithelial cells, we found that chronic exposure to IL-1 β significantly impaired cAMP accumulation and chloride efflux in response to PGE₂.²¹ This effect was accompanied by COX-2 induction and was abolished if cells were treated with selective COX-2 inhibitors. Mechanistic studies showed that this effect was mediated by downregulation of EP4 prostanoid receptors and adenylyl cyclase. The fact that adenylyl cyclase was downregulated suggests that the response to other receptors linked to this pathway would also be impaired. Collectively, these observations suggest that IL-1 β impairs cAMP and chloride responses via an autocrine loop involving COX-2 induction and production of endogenous PGE₂. This might further impair the already defective chloride flux in CF and exacerbate the abnormalities in the composition of the airway surface liquid that occur as a result of CFTR dysfunction, promoting further infection and inflammation.

WHAT ARE THE IMPLICATIONS OF THESE FINDINGS?

The COX inhibitor ibuprofen has been shown to delay the progression of lung disease in children with CF.¹⁵ Ibuprofen is a non-selective COX inhibitor which acts on both COX-1 and COX-2. Studies in healthy volunteers showed that it inhibits 71.4% of COX-2 activity and 88.7% of COX-1 activity.²² Selective COX-2 inhibitors might be more effective, but the recent worries concerning the cardiovascular safety of this class of drugs tempers enthusiasm. Interestingly, glucocorticoids prevent COX-2 induction and this may contribute to their beneficial effects in CF. Further work is needed to examine how inflammatory genes such as COX are regulated in CF, and how these changes impact on disease pathophysiology.

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