

LETTERS TO THE EDITOR

Cyclo-oxygenase

In a recent editorial in *Thorax* Mitchell and Belvisi¹ cite the findings of Sousa and colleagues² to support a theory that aspirin induced adverse respiratory reactions are caused by excessive formation of 15-HETE and 15-epilipoxyins due to the acetylation of cyclo-oxygenase 2 (COX-2) in the lungs of aspirin sensitive asthmatic (ASA) patients by aspirin. Sousa and colleagues reported greater immunoreactivity for the cytokine inducible isoform of COX-2 in the bronchial biopsy specimens of asthmatic patients compared with normal subjects, suggesting a role for COX-2 derived pro-inflammatory prostanooids in the asthmatic airway.² This group also reported a greater proportion of COX-2 immunostaining localised to submucosal mast cells and eosinophils in bronchial biopsy specimens of ASA patients compared with those of non-aspirin sensitive asthmatic (NASA) patients.²

The paradigm put forward by Mitchell and Belvisi has a number of internal contradictions and is not supported by recent experimental evidence. Firstly, Sousa and colleagues found that the overall expression of COX-2 was, in fact, similar in ASA and NASA biopsy specimens,² a finding supported by our group.³ Thus, there is no reason why aspirin induced synthesis of 15-HETE or 15-epilipoxyins should be abnormal in the ASA lung. Secondly, 15-HETE produced by aspirin acetylation of COX-2 is predominantly or exclusively the 15R-HETE stereoisomer, which is biologically inactive.⁴ Thirdly, the active isomer 15S-HETE and its metabolites are extremely weak bronchoconstrictors, while it is well established that adverse respiratory reactions to aspirin and other NSAIDs are caused by the more potent bronchoconstrictors, cysteinyl leukotrienes (cys-LTs), as such reactions are blocked by specific leukotriene synthesis inhibitors and cys-LT receptor antagonists.⁵ Fourthly, and most critically, among the NSAIDs only aspirin covalently acetylates COX-2 and generates 15-HETE, yet patients with ASA show extensive cross sensitivity to other NSAIDs which do not share this property.⁶ The mechanism proposed by Mitchell and Belvisi¹ can therefore play only a negligible pathobiological role in adverse respiratory reactions to aspirin itself, and would not appear to be relevant to similar reactions to other NSAIDs.

The currently accepted model of ASA instead suggests that non-selective NSAIDs including aspirin trigger adverse reactions by releasing the cys-LT synthetic pathway from the suppressive effects of endogenous prostaglandin (PG)E₂, probably synthesised by constitutive COX-1, not by inducible COX-2.⁶⁻⁸ It is agreed that there is no difference in COX-1 expression between ASA, NASA, and normal bronchial biopsy specimens.^{2,3,9} Furthermore, we have recently described a mechanism⁷ which resolves the question of why only patients with ASA, but not those with NASA or normal subjects, produce large amounts of cys-LTs

when the PGE₂ "brake" is removed by NSAIDs.^{8,10} It also explains why these patients have chronically elevated cys-LT production associated with chronic severe asthma, even in the absence of exposure to NSAIDs.¹⁰

The unique terminal enzyme for cys-LT production is LTC₄ synthase, and cloning of the cDNA followed by mutagenic analysis has delineated the amino acid residues central to LTC₄ biosynthesis.^{11,12} The gene for LTC₄ synthase has been localised to chromosome 5q, telomeric to other candidate asthma genes.¹³ We have found that the frequency of cells expressing LTC₄ synthase was five times higher in bronchial biopsy specimens from patients with ASA than in those with NASA, and 19 times higher than in normal biopsy specimens, irrespective of aspirin exposure.³ In contrast, expression of other leukotriene pathway enzymes (5-lipoxygenase and FLAP) and of COX-1 and COX-2 was similar in all subject groups. Increased levels of cys-LTs in the bronchoalveolar lavage (BAL) fluid of unchallenged ASA patients correlated exclusively with the increased number of cells expressing LTC₄ synthase in the bronchial mucosa.³ Following segmental lysine-aspirin challenge, the greater numbers of cells with LTC₄ synthase expression in patients with ASA were associated with rapid increases in cys-LTs in the BAL fluid while the response in patients with NASA was negligible. Increased LTC₄ synthase expression in biopsy specimens from patients with ASA was the only enzyme or cell marker which correlated significantly with bronchial hyperresponsiveness to inhaled lysine-aspirin,³ a definitive clinical measure of aspirin sensitivity. A polymorphism in the LTC₄ synthase gene promoter has since been identified.¹⁴ The A₄₄₄C mutation creates an additional regulatory sequence for the nuclear transcription factor AP-2, perhaps leading to increased expression of LTC₄ synthase protein. By RFLP analysis the polymorphism was shown to be twice as frequent in patients with ASA as in those with NASA or normal subjects, and represents a significant risk factor for aspirin sensitive asthma (odds ratio 3.89, 95% CI 1.57 to 8.98).¹⁴

Together these studies suggest that genetic polymorphisms leading to overexpression of LTC₄ synthase in the bronchial wall of patients with ASA cause chronic overproduction of cys-LTs, which is exacerbated when non-selective NSAIDs suppress the endogenous PGE₂ "brake" generated by COX-1 activity. In contrast to the ASA lung, relatively low expression of LTC₄ synthase precludes clinically significant reactions to NSAIDs in the airways of patients with NASA and those of normal subjects. It is possible that further studies may lead to genetic approaches to identify individuals at risk of aspirin induced reactions, which in turn would aid the targeting of anti-leukotriene drug therapy to this patient group.

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- Mitchell JA, Belvisi MG. Too many COX (cyclo-oxygenase) spoil the broth: aspirin-sensitive asthma and 5-lipoxygenase. *Thorax* 1997;52:933-5.
- Sousa AR, Pfister R, Christie PE, et al. Enhanced expression of cyclo-oxygenase isoform 2 (COX-2) in asthmatic airways and its cellular distribution in aspirin-sensitive asthma. *Thorax* 1997;52:940-5.
- Cowburn A, Sladek K, Soja J, et al. Overexpression of leukotriene C₄ synthase in bronchial biopsies of aspirin-intolerant asthmatics. *J Clin Invest* 1998;101:834-46.
- Lecomte M, Laneuville O, Ji C, et al. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. *J Biol Chem* 1994;269:13207-15.
- Israel E, Fischer AR, Rosenberg MA, et al. The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am Rev Respir Dis* 1993;148:1447-51.
- Szczeklik A. The cyclooxygenase theory of aspirin-induced asthma. *Eur Respir J* 1990;3:588-93.
- Bianco S, Robuschi M, Pettrini G, et al. Efficacy and tolerability of nimesulide in asthmatic patients intolerant to aspirin. *Drugs* 1993;46(Suppl 1):115-20.
- Sestini P, Armetti L, Gambaro G, et al. Inhaled PGE₂ prevents aspirin-induced bronchoconstriction and urinary LTE₄ excretion in aspirin-sensitive asthma. *Am J Respir Crit Care Med* 1996;153:572-5.
- Demoly P, Jaffuel D, Lequeux N, et al. Prostaglandin H synthase 1 and 2 immunoreactivities in the bronchial mucosa of asthmatics. *Am J Respir Crit Care Med* 1997;155:670-5.
- Christie PE, Tagari P, Ford-Hutchinson AW, et al. Urinary leukotriene E₄ concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991;143:1025-9.
- Lam BK, Penrose JF, Freeman GJ, et al. Expression cloning of a cDNA for human leukotriene C₄ synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A₄. *Proc Natl Acad Sci USA* 1994;91:7663-7.
- Lam BK, Penrose JF, Xu K, et al. Site-directed mutagenesis of human LTC₄ synthase. *J Biol Chem* 1997;272:13923-8.
- Penrose JF, Spector J, Baldasaro M, et al. Molecular cloning of the gene for human leukotriene C₄ synthase: organization, nucleotide sequence, and chromosomal localization to 5q35. *J Biol Chem* 1996;271:11356-61.
- Sanak M, Simon HU, Szczeklik A. Leukotriene C₄ synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997;350:1599-600.

AUTHORS' REPLY We are very pleased that four such eminent scientists as Professors Sampson, Holgate, Austen and Szczeklik took the time to respond to our recent editorial in *Thorax*. Our editorial¹ was written to accompany a paper by Sousa and colleagues² showing that COX-2 expression is increased in airway biopsy specimens from asthmatic patients—both aspirin sensitive and aspirin tolerant—when compared with levels in samples from healthy volunteers. Moreover, Sousa *et al* showed that a much greater proportion of mast cells contained COX-2 in tissue from aspirin sensitive patients than from aspirin tolerant patients. This work suggests that COX-2 is a feature of asthma, an observation which confirms the hypothesis made by our group in several papers.^{3,4} Moreover, the differential expression of COX-2 reported by Sousa suggests that this form may have relevance for aspirin sensitivity. In our editorial we have highlighted a phenomenon which occurs when COX-2 is exposed to aspirin—the shunting of products to 15-HETE and further, via 5-LO, to 15-epilipoxyins. However, Professor Sampson and colleagues clearly do not agree that this pathway has any relevance for aspirin induced asthma. We would like to take this

opportunity to clarify some of the points we have made and to address directly the comments made by Sampson *et al.*

Firstly, it is suggested that since Sousa and colleagues found that the overall expression of COX-2 was the same for aspirin sensitive and aspirin tolerant asthmatics, COX-2 can have no role in aspirin sensitive asthma, a notion that is supported by a recently published study by Sampson and colleagues.⁵ This is true; however, Sousa showed a sixfold increase in the number of mast cells expressing COX-2 in aspirin sensitive compared with aspirin tolerant patients. Since cellular location of COX enzymes dictates the products produced and overall effect, we submit, along with Sousa, that this finding is of significant importance. Unfortunately there are clear differences in the results presented by Sousa and those recently published by Sampson *et al.*⁵ Perhaps we must wait for further studies on this subject to have a full appreciation of the differences in COX-2 expression in the airways of asthmatic and normal individuals. It is also suggested that the aspirin induced formation of 15-HETE is predominately in the R form, which is biologically inactive. This point is also true, but aspirin generated 15-HETE is further metabolised by 5-LO to a novel group of metabolites—the 15 epilipoxins⁶—the biological activity of which is not known. Indeed, our editorial clearly showed that it is the involvement of the 5-LO pathway that justifies a role for aspirin generated products in asthmatic symptoms. The final point made by Sampson and colleagues was particularly important. They illustrate the fact that aspirin modification of COX-2 to form 15-HETE is a feature of aspirin and not of other non-steroidal anti-inflammatory drugs (NSAIDs).⁷ Aspirin sensitive patients are thought to respond to all NSAIDs,⁸ a fact that we have freely stated throws doubt on the “COX-2–15HETE” hypothesis. However, it remains the case that the vast majority of studies (including the recent study by Sampson and colleagues) of the severity and mechanisms of aspirin induced asthma use aspirin and not other NSAIDs as a challenge. This approach would be improved if less complex and more potent NSAIDs such as ibuprofen, indomethacin, or diclofenac were used in such studies, at least in addition to aspirin.

In the letter by Sampson and colleagues a new hypothesis is put forward for the mechanisms involved in aspirin sensitive asthma. These authors have recently published new and exciting observations showing that aspirin sensitive individuals express elevated levels of LTC₄ synthase in airway cells. They hypothesise that aspirin sensitive patients express high levels of LTC₄ synthase and that, when the “PGE₂ brake” is removed by NSAIDs, LTC₄ is produced in abundance. It is most unfortunate that this paper was not published at the time we wrote our editorial as we would, of course, have discussed this new hypothesis in the light of data presented by Sousa. However, there are still important questions that require attention before we can be confident that the question of aspirin sensitive asthma is fully addressed. For example, Sampson *et al.* show in their study that aspirin sensitive asthmatics express five times higher levels of LTC₄ synthase than aspirin tolerant asthmatics, and 20 times higher levels than normal individuals. For this new hypothesis to have importance we would assume that

LTC₄ is rate limiting in the production of adenylyl leukotrienes in tolerant asthmatics. This may well be the case; however, the levels of urinary LTE₄ excreted by asthmatics (aspirin sensitivity status not addressed) challenged with allergen (150–1816 pg/ml of creatine)⁹ are in line with those observed in aspirin sensitive patients challenged with aspirin (300–1500 pg/mg creatine).¹⁰ Perhaps the asthmatic patients who develop aspirin sensitivity not only have defects in the expression of LTC₄ synthase but also in the number of braking mechanisms available to suppress its release.

Clearly, the past and current contributions of Professors Sampson *et al.* are of the greatest importance and we are pleased that our editorial has initiated debate on the subject.

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- Mitchell JA, Belvisi MG. Too many COX (cyclo-oxygenase) spoil the broth: aspirin-sensitive asthma and 5-lipoxygenase. *Thorax* 1997;52:933–5.
- Sousa A, Pfister R, Christie PE, *et al.* Enhanced expression of cyclo-oxygenase isoenzyme 2 (COX-2) in asthmatic airways and its cellular distribution in aspirin sensitive asthma. *Thorax* 1997;52:940–5.
- Mitchell JA, Belvisi M, Akaraseenont P, *et al.* Induction of cyclo-oxygenase-2 by cytokines in human pulmonary epithelial cells: regulation by dexamethasone. *Br J Pharmacol* 1994;113:1008–14.
- Belvisi MG, Saunders M, Hirst S, *et al.* Induction of cyclo-oxygenase-2 in human airway smooth muscle: relevance for airway inflammation. *Br J Pharmacol* 1997;120:910–6.
- Cowburn AS, Sladek K, Soja J, *et al.* Overexpression of leukotriene C₄ synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998;101:834–46.
- Claria D, Sherhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by non-steroidal anti-inflammatory drugs. *J Biol Chem* 1993;268:6610–4.
- Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other human endothelial cell-leukocyte interactions. *Proc Natl Acad Sci USA* 1995;92:9475–9.
- Szczeklik A, Gryglewski JR, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. *BMJ* 1975;1:67–9.
- Manning PJ, Rokach J, Malo JL, *et al.* Urinary leukotriene E₄ levels during early and late asthmatic responses. *J Allergy Clin Immunol* 1990;86:211–20.
- Christie PE, Tagari P, Ford-Hutchinson A, *et al.* Urinary leukotriene E₄ concentration increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991;143:1025–9.

Intrapleural streptokinase

Recent publications have advocated the use of intrapleural streptokinase administration in the treatment of empyema complicating community acquired pneumonia.^{1–3} Few complications have previously been described and the treatment appears suitable for most patients. We report two patients with empyema after cardiac surgery in whom admin-

istration of intrapleural streptokinase resulted in life threatening haemorrhage.

A 77 year old man presented six weeks after mitral valve repair with cough, fever and shortness of breath. Chest ultrasound demonstrated a loculated pleural effusion and a percutaneous diagnostic sample was consistent with a diagnosis of empyema. A pigtail catheter was inserted using ultrasound guidance and 250 000 IU of intrapleural streptokinase was instilled twice a day. Two days later he collapsed with haemorrhage into the chest drain. Resuscitation required a six unit blood transfusion and insertion of a 28 F intercostal drain. The patient subsequently made a complete recovery.

A 50 year old man presented with a community acquired pneumonia and loculated empyema nine months after mitral valve repair. A pigtail drain was inserted under ultrasound guidance and streptokinase was administered twice a day. Following three days of treatment the drain was removed but shortly afterwards he collapsed with marked breathlessness. A chest radiograph demonstrated reaccumulation of the effusion and a 28 F intercostal drain was inserted which immediately drained 1500 ml of blood. Surgical decortication and evacuation of the haematoma was performed.

We have observed life threatening haemorrhage in two patients with empyema treated with intrapleural streptokinase. Both patients had recently undergone cardiac valve surgery. We postulate that empyema under these circumstances may represent a distinct, less organised pathological process with a lower fibrin content. We advise particular caution when treatment with intrapleural streptokinase is considered for patients following cardiac surgery.

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- Intrapleural streptokinase for empyema? *Drug Ther Bull* 1997;35:95.
- Taylor R, Rubens M, Pearson M, *et al.* Intrapleural streptokinase in the management of empyema? *Thorax* 1994;49:856–9.
- Davies R, Trail Z, Gleeson F. Randomised controlled trial of intrapleural streptokinase for community acquired pleural infection. *Thorax* 1997;52:416–21.

NOTICE

Pharmacology of Asthma

A course on the pharmacology of asthma will be held at the Imperial College School of Medicine at the National Heart & Lung Institute in collaboration with the Royal Brompton Hospital on 23–26 November 1998. For further information please contact the Postgraduate Education Centre, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, UK. Tel:0171 351 8172. Fax: 0171 376 3442.