# LETTERS TO THE EDITOR

## Cyclo-oxygenase

In a recent editorial in *Thorax* Mitchell and Belvisi[1] cite the findings of Sousa and colleagues[2] to support a theory that aspirin induced adverse respiratory reactions are caused by excessive formation of 15-HETE and 15-epilipoxins 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 prostanoids 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-epilipoxins 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 Belvisi[1] 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)$E_2$, 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 mechanism[3] 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 $PGE_2$ "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 $LTC_4$ synthase, and cloning of the cDNA followed by mutagenic analysis has delineated the amino acid residues central to $LTC_4$ biosynthesis.[11 12] The gene for $LTC_4$ synthase has been localised to chromosome 5q, telomeric to other candidate asthma genes.[13] We have found that the frequency of cells expressing $LTC_4$ 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 $LTC_4$ synthase in the bronchial mucosa.[3] Following segmental lysine-aspirin challenge, the greater numbers of cells with $LTC_4$ 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 $LTC_4$ 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 $LTC_4$ synthase gene promoter has since been identified.[14] The $A_{444}C$ mutation creates an additional regulatory sequence for the nuclear transcription factor AP-2, perhaps leading to increased expression of $LTC_4$ 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 $LTC_4$ 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 $PGE_2$ "brake" generated by COX-1 activity. In contrast to the ASA lung, relatively low expression of $LTC_4$ 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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1 Mitchell JA, Belvisi MG. Too many COX (cyclo-oxygenase) spoil the broth: aspirin-sensitive asthma and 5-lipoxygenase. *Thorax* 1997;**52**:933–5.
2 Sousa AR, 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.
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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 editorial[1] was written to accompany a paper by Sousa and colleagues[2] 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 masts 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.[1 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-epilipoxins. 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 colleges.[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 epilipoxins[6]—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 $LTC_4$ synthase in airway cells. They hypothesise that aspirin sensitive patients express high levels of $LTC_4$ synthase and that, when the "$PGE_2$ brake" is removed by NSAIDs, $LTC_4$ 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 $LTC_4$ synthase than aspirin tolerant asthmatics, and 20 times higher levels than normal individuals. For this new hypothesis to have importance we would assume that $LTC_4$ is rate limiting in the production of adenyl leukotrienes in tolerant asthmatics. This may well be the case; however, the levels of urinary $LTE_4$ 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 $LTC_4$ 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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1 Mitchell JA, Belvisi MG. Too many COX (cyclo-oxygenase) spoil the broth: aspirin-sensitive asthma and 5-lipoxygenase. *Thorax* 1997;**52**:933–5.
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## 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 administration 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. Resucitation 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 aquired 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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# 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.