The role of thromboxane A₂ in the pathogenesis of airway hyperresponsiveness

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Airway hyperresponsiveness to a variety of bronchoconstrictor mediators and physical stimuli, such as exercise, is present in patients with asthma [1]. Inhalation of inflammatory stimuli such as allergens [2] or occupational sensitizing agents [3] in sensitized subjects, the atmospheric pollutant ozone [4], or the development of viral upper respiratory tract infections [5] can cause transient airway hyperresponsiveness, which can last days, weeks or occasionally months [6] and is associated with worsening symptoms of asthma [7].

Investigation of the pathogenesis of airway hyperresponsiveness has been most easily carried out in animals or humans by studying stimuli known to cause airway hyperresponsiveness. This is because administration of these stimuli can be carefully controlled in a laboratory setting. Those most extensively studied have been the inhaled allergens, toluene diisocyanate (TDI) or ozone both in humans [6, 8, 9], and in several animal models [10–13]. There are, however, several problems with this approach. For example, the mechanisms of airway hyperresponsiveness, even to the same stimulus, appear to differ between species [14, 15]. Also, the mechanisms causing acute airway hyperresponsiveness may be different to those causing persistent airway hyperresponsiveness in asthma. Thromboxane A₂ (Txₐ₂), may be an important mediator in the pathogenesis of airway hyperresponsiveness in animal models, and allergic responses as well as transient and persistent airway hyperresponsiveness in humans. This article will review the evidence that Txₐ₂ is involved in these responses.

Thromboxane A₂

Thromboxane A₂ (Txₐ₂) is a cyclooxygenase product of arachidonate metabolism. The compound was initially described by Piper and Vane [16] in 1969 as contracting rabbit aorta in a bioassay, and was first characterized and named by HAMBERG et al. [17] in 1975. Txₐ₂ is known to be both a potent constrictor of smooth muscle, particularly vascular smooth muscle, and to cause platelet aggregation. Txₐ₂ was originally described as being released from platelets [17], but is now known to be released from other cells, including macrophages and neutrophils [18].

The biological half-life of Txₐ₂ is very short (approximately 30 s); therefore, implicating Txₐ₂ in disease processes has depended on measurement of its more stable metabolic Thromboxane B₂ (TxB₂) in biological fluids; on the use of the stable endoperoxides U44069 or U46619, which mimic most of the biological effects of Txₐ₂, and have been used as Txₐ₂ analogues; and on the use of inhibitors of Txₐ₂ synthesis and antagonists of the Txₐ₂ receptor. Using these techniques, Txₐ₂ has been implicated in the pathogenesis of airway hyperresponsiveness in dogs [19–22] and primates [23]; of the late cutaneous response to intradermal allergen [24] in humans; of the immediate response to inhaled allergen in dogs [25]; of the late asthmatic response after inhaled allergen in humans [26]; and of airway hyperresponsiveness in asthmatic subjects [27].

Txₐ₂ in the pathogenesis of airway hyperresponsiveness

Animal models

Studies examining the role of cyclooxygenase products of arachidonate in the pathogenesis of airway hyperresponsiveness were initially carried out using the cyclooxygenase inhibitor, indomethacin. These studies were performed in dogs with airway hyperresponsiveness after inhaled ozone. Indomethacin did not alter baseline airway responsiveness to inhaled acetylcholine, but did prevent the development of airway hyperresponsiveness after inhaled ozone [28]. Despite the absence of airway hyperresponsiveness, the magnitude of the inflammatory response, as measured by the numbers of neutrophils in the airway epithelium, was not altered by indomethacin. This suggested that a cyclooxygenase product was not responsible for the chemotaxis of acute inflammatory cells into the airways after inhaled ozone; however, a cyclooxygenase product was released during the inflammatory response which caused airway hyperresponsiveness. Subsequently, a reputed combined cyclooxygenase and lipoxygenase inhibitor, BW775c was also demonstrated to prevent the development of airway hyperresponsiveness after inhaled ozone in dogs [29]. Inhibition of cyclooxygenase by indomethacin also prevents the development of airway hyperresponsiveness.
in other species; for example, after C₄₄ des arg in rabbits [30], and after inhaled allergen in sheep [31]. Other investigators, however, demonstrated that BW775c, but not indomethacin, prevents airway hyperresponsiveness after inhaled ozone in guinea-pigs [32], suggesting that a lipoxygenase rather than a cyclooxygenase product was causing airway hyperresponsiveness in this species.

The cyclooxygenase product causing airway hyperresponsiveness after inhaled inflammatory stimuli in these animal models is most likely TxA₂. This is based on three lines of evidence. Firstly, a TxA₂ synthetase inhibitor prevents the development of airway hyperresponsiveness. Secondly, increased levels of TxB₂, the metabolite of TxA₂, can be measured in bronchoalveolar lavage (BAL) fluid from dogs with airway hyperresponsiveness after leukotriene B₄ (LTB₄). Thirdly, TxA₂ mimetics, such as U46619, can cause airway hyperresponsiveness. A TxA₂ synthetase inhibitor, OKY 046 has been demonstrated to effectively inhibit the development of acetycholine airway hyperresponsiveness after inhaled allergen [19], ozone [20], LTB₄ [21], and platelet activating factor (PAF) [22] in dogs, without changing baseline acetycholine airway responsiveness or the influx of inflammatory cells after inhalation of each of these stimuli. These results implicated TxA₂ in causing airway hyperresponsiveness, but not in causing chemotaxis of inflammatory cells into the airways. One problem with interpretation of these results, however, is that inhibition of TxA₂ synthesis in other systems increases levels of other prostanoids, such as prostaglandin I₂ (PGI₂). Therefore, the inhibition of airway hyperresponsiveness after these stimuli may reflect the production of a protective prostanoïd rather than inhibition of TxA₂.

Increased levels of TxB₂ have been demonstrated in BAL fluid from dogs with airway hyperresponsiveness after inhaled LTB₄ [21]. The increase in TxB₂ was correlated with neutrophil counts in BAL. Significant increases were also seen in levels of prostaglandins E₂ and F₂ after inhaled LTB₄, OKY 046 inhibited not only the development of airway hyperresponsiveness, but also the rise in levels of TxB₂ in BAL, without altering the increase in prostaglandins E₂ and F₂, or the influx of neutrophils after inhaled LTB₄. The increase in TxB₂ in BAL may, however, be specific to LTB₄ challenge as no increase in TxB₂ could be found in BAL fluid from Ascaris-sensitive dogs challenged with allergen, although there was a large increase in prostaglandin D₂ (PGD₂) and histamine [33].

The final piece of evidence is the demonstrated that inhaled subthreshold concentrations of the TxA₂ mimetic, U46619, can increase airway responsiveness to acetycholine in dogs [20]. This effect was not seen with another contractile agonist, histamine. These studies all suggest that TxA₂ release in the airways is important in the pathogenesis of airway hyperresponsiveness after inhalation of inflammatory stimuli such as ozone, allergen, PAF and LTB₄ in dogs. Studies in primates have demonstrated that another TxA₂ mimetic, U44069, increases airway responsiveness to acetycholine and that this effect was abolished by the TxA₂ receptor antagonist, L655, 240 [23].

Mechanism of action of TxA₂ in the airways

The mechanism by which TxA₂ causes airway hyperresponsiveness is not yet known, but possible mechanisms include presynaptic modulation of acetycholine release, or an effect on airway smooth muscle.

TxA₂ was demonstrated to modulate acetycholine release in airways initially by Munoz et al. [34] using the TxA₂ mimetic U46619, which increased the response to field stimulation in trachealis muscle. This effect lasted more than 60 min. No increase in the response to exogenous acetycholine by U46619 was demonstrated, suggesting that the augmentation was occurring presynaptically, through increased acetycholine release in response to field stimulation. Further support for this hypothesis was provided by Tamaki et al. [35], who demonstrated that aggregated platelets in an organ bath released TxA₂. The TxA₂ transiently increased the responses to field stimulation and this effect was prevented by a TxA₂ receptor antagonist. Once again, the response to an exogenous cholinergic agonist was not altered by the released TxA₂.

The increased response to field stimulation may not, however, necessarily be caused by increased amounts of acetycholine released from the nerve terminal. For example, Serio and Daniel [36] have demonstrated that U46619 increases the response to electrical field stimulation in canine trachealis, an effect that is antagonized by a TxA₂ receptor antagonist. The effect on electrical field stimulation was not seen with leukotriene B₄ or D₄ or prostaglandin D₂. The effect of U46619, however, was not associated with increases in the amplitude or duration of associated excitatory junctional potentials (EJPs), but rather with an increase in the number of secondary oscillations which follow the EJP. These results suggest that TxA₂ may not enhance the initial amount of acetycholine release per se, but rather the duration of release or may improve the propagation of activity through the muscle.

Presynaptic modulation of acetycholine release may also be altered in dogs with airway hyperresponsiveness after inhaled ozone [37]. When trachealis muscle from dogs with airway hyperresponsiveness in vivo was studied in the organ bath, the response to electrical field stimulation was increased when compared to control dogs. By contrast, the responses to exogenous acetycholine were no different between the two groups of dogs. These results suggested that trachealis muscle itself was no different in dogs with airway hyperresponsiveness after ozone, but that the amounts of acetycholine being released in response to nerve stimulation was increased. These results are consistent with the known effects of TxA₂ on acetycholine release in canine trachealis already discussed. Therefore, throm-
boxane, released as part of the airway inflammatory response after inhaled ozone, may be responsible for the increased response to nerve stimulation in the ozone treated tissues.

Recent studies in our laboratories have confirmed the results of WARKINS et al. [37] that the responses to electrical field stimulation are increased in ozone treated dogs when compared to control dogs [38]. However EJPs, whether measured by the single or double sucrose gap, were no different between the two groups of dogs. These results were qualitatively similar to the results of SERRIO and DANIEL [36] on the effects of U46619 on the responses to field stimulation. These results suggest that something other than an increase in the initial amount of acetylcholine released is responsible for the increased response to field stimulation in muscle strips from dogs with airway hyperresponsiveness in vivo.

TxA₂ can act on specific receptors on airway smooth muscle. This is because U44069 constricts airway smooth muscle, an effect which is abolished by TxA₂ receptor antagonists [23, 36]. There is, however, no evidence that TxA₂ can increase responsiveness of smooth muscle to other constrictor agonists in vitro.

Studies in human subjects

Studies examining the importance of specific mediators in the pathogenesis of asthma are complicated, when compared to studies in animal models, by the lack of potent, specific mediator antagonists, and by the difficulties in measuring the mediator or its metabolite at its site of action in the airways. For example, indomethacin, which has been extensively used to implicate cyclooxygenase products in the pathogenesis of disease, inhibits the production of not only potentially harmful mediators such as TxA₂ and prostaglandin D₂, but also potentially useful mediators in asthmatic airways, such as prostaglandin E₂ [39]. These difficulties have been partly overcome in studies examining the response to allergen in the skin.

TxA₂ has been implicated in the pathogenesis of allergen-induced late cutaneous response in human subjects. GROENENBERG et al. [40] have demonstrated partial inhibition of the late cutaneous response to antigen after pretreatment with indomethacin. Also, an increase in TxB₂ levels has been demonstrated in skin blister fluid during the late cutaneous response, 6 h after injection of allergen [41]. These investigators have also shown that a TxA₂ synthesis inhibitor, dazoxiben, inhibits the late cutaneous response [24]. Thus, it appears that TxA₂ may be responsible for some of the manifestations of the late cutaneous, but not the early cutaneous response, after injected allergen.

TxA₂ is released from lung tissue in allergic asthmatic subjects after stimulation with specific allergen [42]. However, sulphidopeptide leukotrienes, released at the same time, appear to be more important in causing contractile responses after stimulation with allergen in vitro [42].

Attempts to measure TxB₂ during asthma or following allergen challenge have led to conflicting results. SHERBARD et al. [26] have demonstrated increased levels of TxB₂ in plasma following allergen challenge. However, plasma TxB₂ measurements must be viewed with caution because of the possibility of local platelet generation of TxB₂, and measurements should be confirmed by assaying the free, 2, 3-dinor-metabolite of TxB₂, which cannot come from platelet activation alone. In a small series of atopic asthmatics, challenged with allergen bronchoscopically, TxB₂ levels were not elevated when compared to a sham challenge. However, in a patient with status asthmaticus, increased levels of TxB₂ in lavage fluid was found, but this could have been due to ex vivo generation of TxB₂ from alveolar macrophages. In a larger series of patients with either acute asthma or undergoing allergen challenge, urinary levels of TxB₂ and 2, 3-dinor-TxB₂ were measured. In both groups the levels of these mediators were within the normal range. This lack of evidence for TxA₂ generation does not rule out its importance, as high local levels generated in the region of the smooth muscle may not lead to a significant increase in total body turnover of the metabolite.

Cyclooxygenase products have been implicated in the pathogenesis of allergen-induced early asthmatic [43] as well as late asthmatic responses [44, 45]. This has been done by pretreating subjects with several different cyclooxygenase inhibitors. For example, JOUNNEET et al. [45] reported that pretreatment with indomethacin inhibited the late response in 10 out of 11 subjects studied, without having a major effect on the early response. More recent studies, however, have not confirmed these observations on either allergen-induced early or late responses. Pretreatment with indomethacin (100 mg·day⁻¹) administered in a double-blind, random- ized, cross-over fashion did not influence either the early or late asthmatic responses [46]. These results suggested that cyclooxygenase products, including TxA₂, were not important mediators in causing allergen-induced asthmatic responses.

There is evidence which implicates a cyclooxygenase product, possibly TxA₂, in the pathogenesis of airway hyperresponsiveness in asthmatic subjects. Indomethacin significantly inhibits the development of airway hyperresponsiveness after inhaled allergen in allergic subjects [46]. This suggests that a cyclooxygenase product is involved in the pathogenesis of airway hyperresponsiveness after inhaled allergen. In addition, the TxA₂ synthetase inhibitor, OKY 046, administered orally, reduces acetylcholine airway hyperresponsiveness in stable asthmatic subjects (although these studies were uncontrolled), while a lipooxygenase inhibitor had no effect in these subjects [27]. Thus, TxA₂ may be an important mediator in the pathogenesis of airway hyperresponsiveness in stable asthma or after inhaled allergens. Recent studies, however, have examined the effect of a thromboxane synthetase inhibitor, CGS 12970, on airway responsiveness measured 4 h and 6 h after allergen challenge. CGS 12970 had no effect on either the early or late responses after inhaled allergen. In addition, there was no effect on airway
hyperresponsiveness to inhaled histamine measured at 4 h and 6 h post-allergen [47].

Chronic smokers also have an increase in airway response to inhaled spasmogens, which may also be due in part to airway inflammation. It has been observed that smokers have a nearly two-fold increase in urinary 2, 3-dinor-TxB 2 [48]. In a group of smokers with airway hyperresponsiveness the urinary levels of 2, 3-dinor-TxB 2 were no different from a group of smokers with normal airway responsiveness, nor was there any correlation between the level of airway responsiveness and excretion of the metabolite. Furthermore, treatment with the cyclooxygenase inhibitor, flurbiprofen 150 mg for 3 days did not change airway responsiveness despite abolishing the excretion of the metabolite [49]. It appears unlikely, therefore, that TxA 2 is responsible for the airway hyperresponsiveness found in some smokers.

Conclusions

The studies described have suggested that TxA 2 is released from inflammatory cells in the airways and may be important in causing airway hyperresponsiveness after a number of inflammatory stimuli in dogs. The action of TxA 2 is through a presynaptic effect on airway cholinergic nerves and also possibly a postsynaptic effect on airway smooth muscle. A cyclooxygenase product of arachidonic metabolism (possibly TxA 2), is also important in causing airway hyperresponsiveness in other species, such as rabbits and sheep.

In human subjects, the role of TxA 2 in causing airway hyperresponsiveness is much less clear. A cyclooxygenase product appears to be involved in allergen-induced airway hyperresponsiveness. Attempts to measure abnormal TxA 2 production in asthma has so far been unsuccessful. Studies with two thromboxane synthetase inhibitors have provided conflicting results. However, synthetase inhibitors may not be the ideal drugs to use, as reactive endoperoxides may be released during synthetase inhibition; therefore, thromboxane receptor antagonists need to be studied. It is clear that a large amount of research needs to be done to confirm and extend these observations before the precise role of thromboxane in the pathogenesis of asthma is clarified.

References

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