Leukotriene D₄- and prostaglandin F_{2α}-induced airflow obstruction and airway plasma exudation in guinea-pig: role of thromboxane and its receptor

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1 We studied the effects of a thromboxane A₂ receptor (TP receptor) antagonist, ICI-192,605 (0.5 mg kg⁻¹, i.v.) and a selective thromboxane (Tx) synthetase inhibitor, OKY-046 (30 mg kg⁻¹, i.v.), on airway responses induced by leukotriene D₄ (LTD₄; 0.2 nmol) or prostaglandin F_{2α} (PGF_{2α}; 20 nmol) instilled via the airways route to anaesthetized guinea-pigs. For a comparison, airway responses to a TxA₂-mimetic, U-46619 (0.02 nmol) were also studied. We measured both lung resistance (R_L) to monitor airflow obstruction, and extravasation of Evans Blue dye to quantify airway plasma exudation.

2 Instilled LTD₄ into the tracheal lumen induced an immediate peak and subsequently persistent increase in R_L and produced a large amount of extravasation of Evans Blue dye at all airway levels. Both ICI-192,605 and OKY-046 significantly attenuated the persistent increase in R_L following the immediate response and reduced LTD₄-induced extravasation of Evans Blue dye in the trachea and proximal intrapulmonary airway. Instilled LTD₄ produced significant increases in immunoreactive TxB₂ in bronchoalveolar lavage fluid obtained 1.5 min after instillation of LTD₄.

3 Instilled PGF_{2α} into the tracheal lumen induced an immediate increase in R_L which peaked at approximately 15 s. We also observed a delayed sustained increase in R_L, reaching a second peak at approximately 4 min. PGF_{2α} produced small but significant increases in the amount of Evans Blue dye at all airway levels. As with PGF_{2α}, instillation of U-46619 produced a biphasic increase in R_L and extravasation of Evans Blue dye. The potency of PGF_{2α} in inducing these airway responses was about 1000 times less than U-46619. ICI-192,605 abolished both the immediate and the delayed increase in R_L after PGF_{2α}, and also blocked PGF_{2α}-induced extravasation of Evans Blue dye. However, OKY-046 had no inhibitory effects on these responses.

4 We conclude that airflow obstruction and airway plasma exudation induced by instilled LTD₄ is, in part, mediated via TxA₂ generation and subsequent activation of TP-receptors. On the other hand, instilled PGF_{2α}, while inducing similar responses, does so primarily by direct activation of TP receptors, rather than via TxA₂ generation.

Keywords: Asthma; airway oedema; arachidonic acid; vascular permeability

Introduction

The role of thromboxane A₂ (TxA₂) in bronchial asthma remains unclear, despite several studies designed to evaluate this (Barnes *et al.*, 1988; O'Byrne & Manning, 1992). Recent *in vivo* studies in guinea-pigs have revealed that a TxA₂-mimetic, U-46619 (9,11-dideoxy methanoepoxy-9α, 11α-prostaglandin F_{2α}), induces airflow obstruction and airway plasma exudation (Lötvall *et al.*, 1992). In addition, TxA₂ may, in this species, be one of the final pathways of airflow obstruction and airway plasma exudation induced by platelet activating factor (Tokuyama *et al.*, 1991) and bradykinin (Arakawa *et al.*, 1992; Kawikova *et al.*, 1992). In guinea-pigs, allergen-induced airflow obstruction is mediated in part via TxA₂ production (Beasley *et al.*, 1989; Arakawa *et al.*, 1993), and an increased level of TxB₂ is found in bronchoalveolar lavage after allergen challenge (Fujimura *et al.*, 1991).

The role of cyclo-oxygenase products in leukotriene D₄ (LTD₄)-induced airway responses in guinea-pigs is controversial. Both Hamel *et al.* (1982) and Birch *et al.* (1988) have suggested that TxA₂ mediates bronchoconstrictor responses to i.v. LTD₄ in guinea-pigs. However, cyclo-oxygenase products were found not to mediate either bronchoconstriction (Hamel *et al.*, 1982) or airway plasma exudation (Woodward *et al.*, 1983) induced by LTD₄ given via the airway route in guinea-pigs.

PGF_{2α} causes airflow obstruction (Birch *et al.*, 1988) and weak airway plasma exudation (Persson, 1992). Bronchoconstriction induced by PGF_{2α} or PGD₂ is mediated through activation of TxA₂ receptors (TP receptors) (Birch *et al.*, 1988). Also, *in vitro* studies have demonstrated that PGF_{2α} acts in part through TP receptors to induce contraction of lung parenchymal strips from guinea-pigs (McKenniff *et al.*, 1991). However, to our knowledge there is no information available concerning the inhibitory effects of TP-receptor antagonists on PGF_{2α}-induced airway plasma exudation.

The aims of this study were first to evaluate whether TxA₂ is involved in airway responses to instilled LTD₄, and secondly, to investigate whether PGF_{2α}-induced airway plasma exudation is mediated through activation of TP receptors. We studied the effects of ICI-192,605, a TP receptor antagonist, and OKY-046, a selective Tx synthetase inhibitor, on both airflow obstruction and airway plasma exudation induced by either LTD₄ or PGF_{2α}. For comparison, we also studied the effects of a TxA₂-mimetic, U-46619, instilled into the tracheal lumen.

Methods

Experiments were performed on male Dunkin Hartley guinea-pigs weighing 450–670 g. The animals were anaesthetized with a 3:2 mixture of ketamine (50 mg ml⁻¹) and xylazine (20 mg ml⁻¹) intramuscularly (1 ml kg⁻¹). Additional anaes-

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thetic was given to an appropriate level of anaesthesia as evidenced by a disappearance of corneal reflex and withdrawal response to foot pad pinching. Anaesthesia was subsequently monitored by these means until the time when suxamethonium was administered to avoid artefacts induced by spontaneous breathing. Thereafter, systemic blood pressure was monitored throughout the experiment. A tracheal cannula (10 mm length and 2.7 mm inner diameter) was inserted into the lumen of the cervical trachea through a tracheostomy, and secured with a suture. A polyethylene catheter was inserted into the left carotid artery to monitor blood pressure with a pressure transducer (model P23XL, Viggo-Spectramed, Helsingborg, Sweden). The right external jugular vein was cannulated for the administration of drugs and Evans Blue dye. All animals were pretreated with suxamethonium (5 mg kg^{-1} , i.v.) to avoid artefacts induced by spontaneous breathing.

Measurement of airway function

Guinea-pigs were placed on a heated blanket (Harvard model 50-7061, Harvard Apparatus Ltd., Edenbridge, Kent) which maintained body temperature at 37°C , with the intratracheal cannula connected to a constant volume mechanical ventilator (Harvard model 50-1718, Harvard Apparatus Ltd.). A tidal volume of 10 ml kg^{-1} and a frequency of 60 breaths min^{-1} was used. Transpulmonary pressure was measured with a pressure transducer (Model FCO40; $\pm 1000 \text{ mmHg}$; Furness Controls Ltd., Bexhill, Sussex), with one side attached to a catheter inserted into the right pleural cavity and the other side attached to a catheter connected to a side port of the intratracheal cannula. The ventilatory circuit had a total volume of 18 ml. Airflow was measured with a pneumotachograph (Model F1L; Mercury Electronics Ltd., Glasgow) connected to a transducer (Model FCO40; $\pm 20 \text{ mmHg}$; Furness Controls Ltd.). The signal from the blood pressure transducer was amplified with an analog preamplifier (Kungbacka mät & reglerteknik AB, Sweden). All signals were digitalized with a 12-bit analog digital board (National Instruments, Austin, Tx, U.S.A.) connected to a Macintosh II computer (Apple computer Inc., Cupertino, U.S.A.) and analyzed with a software (LabView, National Instruments), which was programmed to calculate instantaneously lung resistance (R_L) by the method of von Neergaard & Wirz (1927). Mean blood pressure was also monitored throughout the experiments.

Protocol

The animals were divided into six groups in order to study the effects of pretreatment with ICI-192,605 (0.5 mg kg^{-1} ; Lötvall *et al.*, 1992) or OKY-046 (30 mg kg^{-1} ; Arakawa *et al.*, 1992) on both airway plasma exudation and increase in R_L up to 6 min after instillation of LTD₄ (0.2 nmol ; $n = 7$ and $n = 6$, respectively) or PGF_{2 α} (20 nmol ; $n = 6$ and $n = 4$, respectively), compared to untreated animals (LTD₄, $n = 9$; PGF_{2 α} , $n = 9$). For comparison, we also studied the effects of a TxA₂ mimetic, U-46619 (0.02 nmol , $n = 5$) or vehicle (0.9% NaCl, $n = 6$) instilled into the tracheal lumen of untreated guinea-pigs in two groups (Table 1). Furthermore, we measured TxB₂, a stable metabolite of TxA₂, in bronchoalveolar lavage fluid (BALF) of guinea-pigs 1.5 min after instilled LTD₄ or saline (0.9% NaCl) in another two groups.

Ten minutes after pretreatment with suxamethonium, either ICI-192,605 (0.5 mg kg^{-1}) or OKY-046 (30 mg kg^{-1}) was given intravenously. Five minutes later, or 15 min after suxamethonium (untreated animals), Evans Blue dye (20 mg kg^{-1}) was administered i.v. over 1 min. Another minute later, $100 \mu\text{l}$ of LTD₄ ($2 \mu\text{M}$), PGF_{2 α} ($200 \mu\text{M}$), U-46619 ($0.2 \mu\text{M}$) or vehicle (0.9% NaCl) was instilled into the airways. Airway instillation was performed by flushing $100 \mu\text{l}$ volumes with 1 ml of air, directly into the tracheal lumen via the tracheal cannula. At that time, the animals were disconnected from

the ventilator for a few seconds. Transpulmonary pressure, R_L and mean blood pressure were monitored by the computer.

Determination of plasma extravasation

Six minutes after administration of LTD₄, PGF_{2 α} , U-46619 or vehicle, the animals were disconnected from the ventilator, and the thoracic cavity was opened. A catheter was inserted into the aorta through the left ventricle, and the animal was perfused with 50 ml of 0.9% NaCl to remove dye within the bronchial circulation. After perfusion through this route, another catheter was inserted into the pulmonary artery, and the pulmonary circulation was then perfused with 20 ml of saline. The trachea and lungs were dissected out en bloc, the parenchyma carefully scraped off, and extraneous tissue removed. The trachea, main bronchi and intrapulmonary airways were separated from each other, and the intrapulmonary airways were then divided into two lengthwise equal portions, arbitrarily named proximal and distal. All airway tissues were dried, with a freeze dryer (MicroModulyo, Edwards High Vacuum International, West Sussex) for 24 h. All tissues were weighed dry, and Evans Blue dye was extracted in 2 ml of formamide in a 40°C water bath for 24 h. Absorption at 620 nm was measured in a spectrophotometer (PU8670 VIS/NIR, Philips, Cambridge, U.K.). The extracted Evans Blue dye was quantified by interpolation on a standard curve of dye concentrations in the range of $0\text{--}10 \mu\text{g ml}^{-1}$, and expressed as ng mg^{-1} dry tissue. Evans Blue dye measurement has been previously shown to correlate highly with the extravasation of radiolabelled albumin in guinea-pig airways (Rogers *et al.*, 1989).

Measurement of TxB₂ in BALF

Ninety seconds after instillation of LTD₄ (0.2 nmol) or vehicle (0.9% NaCl), 10 ml kg^{-1} saline at 37°C was instilled through the tracheal cannula and sequentially removed by gentle manual aspiration. The recovered BALF was immediately put into a tube with indomethacin (final concentration, $10 \mu\text{M}$) and centrifuged for 10 min at $400 g$ to separate fluid from cells and debris. The supernatant was stored at -25°C for measurement of TxB₂ concentration. TxB₂ concentration in BALF was measured by radioimmunoassay kit (Amersham, Stockholm, Sweden), using [¹²⁵I] TxB₂ as standard, and an antibody to TxB₂. The activity was counted in a gamma counter (LKB Wallac, Wallac, Sollentuna, Sweden), and the amount of TxB₂ expressed as pM. The percentage cross-reactivity of this kit with leukotriene D₄ was less than 0.35, in preliminary experiments. Briefly, we found an apparent value of TxB₂ of 31 pg ml^{-1} after adding $10,000 \text{ pg LTD}_4$ to the solution.

Drugs and chemicals

The following drugs and chemicals were used: ketamine hydrochloride (Park-Davis S.A., Barcelona, Spain); xylazine chloride (Bayer Sverige AB, Göteborg, Sweden); suxamethonium chloride (KabiVitum AB, Stockholm, Sweden); Evans Blue dye (Aldrich Chemical Co., Milwaukee, U.S.A.); leukotriene D₄ (Sarva, Germany); prostaglandin F_{2 α} , U-46119 (Sigma, St Louis, U.S.A.); ICI-192,605: 4-(Z)-6-(2-*o*-chlorophenyl-4-*o*-hydroxyphenyl-1,3-dioxan-*cis*-5-yl) and OKY-046; (E)-3-[*p*-(4-imidazol-methyl) phenyl]-2-propenoic acid hydrochloride were kindly donated by ICI Pharmaceutical Co. (USA) and Kissei Pharmaceutical Co. (Japan). ICI-192,605 was at first diluted with 100 mM NaHCO_3 to give a concentration of 5 mg ml^{-1} , and further dilutions were made with 0.9% NaCl to give a final concentration of 0.5 mg ml^{-1} (Lötvall *et al.*, 1992). In a previous study, ICI-192,605

administration alone had no effect on baseline R_L or extravasation of Evans Blue dye (Tokuyama *et al.*, 1991). OKY-046 was diluted in saline. All drugs injected intravenously were given at a dose volume of 1 ml kg⁻¹.

Data analysis

Data are given as mean $\pm$ s.e.mean. Non-parametric analysis of variance (Kruskal-Wallis method) was used to determine any significant variance among groups. If a significant variance was found, a Mann-Whitney U-test was used to test for significant differences between individual groups. A P value less than 0.05 was considered significant. Data were analyzed by a Macintosh computer using standard statistical packages (StatView II).

Results

R_L -changes induced by LTD₄, PGF_{2α} and U-46119

There were no significant differences in body weight and baseline R_L between any of the groups studied (Table 1). In untreated animals, instilled LTD₄ (0.2 nmol) produced an immediate and subsequently persistent increase in R_L , with an immediate peak at approximately 45 s (3.5 ± 0.5 cmH₂O ml⁻¹ s) (Figure 1). R_L was significantly higher after LTD₄ than after instillation of saline at all time points. Neither ICI-192,605 nor OKY-046 significantly affected the immediate increase in R_L induced by LTD₄. However, the persistent increase in R_L following the immediate response was significantly attenuated by pretreatment with both ICI-192,605 and OKY-046 at all time points longer than 1.5 min after instillation of LTD₄.

In untreated animals, instilled PGF_{2α} (20 nmol) produced a biphasic increase in R_L , with an immediate peak at approximately 15 s (2.2 ± 0.8 cmH₂O ml⁻¹ s) and a delayed sustained increase in R_L which peaked at approximately 4 min (2.2 ± 0.2 cmH₂O ml⁻¹ s) (Figure 2). R_L was significantly higher after PGF_{2α} than after instillation of saline at all time points. Instillation of U-46619 (0.02 nmol) also induced a biphasic increase in R_L , with similar levels of increased R_L compared with those after PGF_{2α} (Figure 2). ICI-192,605 abolished both the immediate and the delayed sustained increase in R_L induced by PGF_{2α}. However, OKY-046 did not affect these responses (Figure 2).

Evans Blue dye extravasation induced by LTD₄, PGF_{2α} and U-46619

In untreated animals, instillation of LTD₄ (0.2 nmol) caused pronounced extravasation of Evans Blue dye at all airway levels (Figure 3). Both ICI-192,605 and OKY-046 reduced extravasation of dye after LTD₄, with a significant inhibition

Figure 1 Effect of ICI-192,605 (●: 0.5 mg kg⁻¹, $n = 7$) or OKY-046 (▲: 30 mg kg⁻¹, $n = 6$) given i.v. 5 min before instillation of leukotriene D₄ (LTD₄, 0.2 nmol) on the time-course of increased lung resistance (R_L), compared with untreated guinea-pigs given instilled LTD₄ (○: $n = 9$) or saline (□: 0.9% NaCl, $n = 6$). * $P < 0.01$, ** $P < 0.005$ compared to untreated animals given instilled LTD₄.

in trachea ($P < 0.01$ and $P < 0.05$, respectively) and proximal intrapulmonary airways ($P < 0.01$ after ICI-192,605). These values after ICI-192,605 or OKY-046 were, however, still significantly higher than in animals given vehicle instillation. In untreated animals, instillation of PGF_{2α} (20 nmol) caused a small but significant increase in extravasation of Evans Blue dye at all airway levels (Figure 3). U-46619 (0.02 nmol) also produced significant extravasation of Evans Blue dye at all airway levels (Figure 3). The amount of extravasated dye was larger after U-46619 than PGF_{2α}, with a significant difference in trachea ($P < 0.05$). ICI-192,605 significantly blocked extravasation of dye after PGF_{2α} at all airway levels, except for distal intrapulmonary airways. However, OKY-046 did not influence PGF_{2α}-induced extravasation of Evans Blue dye (Figure 3).

Blood pressure-changes induced by LTD₄, PGF_{2α} and U-46619

There were no significant differences in baseline mean systemic blood pressure between any of the groups studied

Table 1 Body weight, baseline data for lung resistance (R_L), mean systemic blood pressure, and percentage change in mean systemic blood pressure after either leukotriene D₄ (LTD₄) or prostaglandin F_{2α} (PGF_{2α})

Group	LTD ₄ (0.2 nmol)					PGF _{2α} (20 nmol)		
	Saline (0.9% NaCl)	U-46619 (0.02 nmol)	Untreated	ICI-192,605 (0.5 mg kg ⁻¹)	OKY-046 (30 mg kg ⁻¹)	Untreated	ICI-192,605 (0.5 mg kg ⁻¹)	OKY-046 (30 mg kg ⁻¹)
Body weight (g)	512 $\pm$ 5	532 $\pm$ 7	612 $\pm$ 12	611 $\pm$ 13	592 $\pm$ 17	523 $\pm$ 15	535 $\pm$ 9	513 $\pm$ 15
Number	$n = 6$	$n = 5$	$n = 9$	$n = 7$	$n = 6$	$n = 9$	$n = 6$	$n = 4$
Baseline R_L (cmH ₂ O ml ⁻¹ s)	0.27 $\pm$ 0.01	0.25 $\pm$ 0.01	0.29 $\pm$ 0.02	0.29 $\pm$ 0.01	0.28 $\pm$ 0.01	0.31 $\pm$ 0.02	0.27 $\pm$ 0.01	0.29 $\pm$ 0.02
Blood pressure baseline (mmHg)	53 $\pm$ 1	52 $\pm$ 2	51 $\pm$ 2	49 $\pm$ 2	50 $\pm$ 3	52 $\pm$ 2	47 $\pm$ 1	47 $\pm$ 3
% change	-10.8 $\pm$ 5.2	-62.3 $\pm$ 2.8	-61.3 $\pm$ 2.0	-64.5 $\pm$ 2.3	-65.8 $\pm$ 2.6	-59.9 $\pm$ 1.4	-14.9 $\pm$ 3.5 *	-56.8 $\pm$ 2.7

Values are expressed as mean $\pm$ s.e.mean.

* $P < 0.001$ as compared to untreated animals given instilled PGF_{2α}.

(Table 1). In untreated animals, instillation of LTD₄ (0.2 nmol) caused an immediate fall and subsequently transient increase in mean systemic blood pressure approximately 1

Figure 2 (a) Effect of ICI-192,605 (■: 0.5 mg kg⁻¹, *n* = 6) or OKY-046 (●: 30 mg kg⁻¹, *n* = 4) given i.v. 5 min before instillation of prostaglandin F_{2α} (PGF_{2α}, 20 nmol) on the time-course of changes in R_L, compared with untreated guinea-pigs given instilled PGF_{2α} (○: *n* = 9). (b) Time course of effect of instilled U-46619 (▲: 0.02 nmol; *n* = 5) or saline (□: 0.9% NaCl, *n* = 6) on R_L in untreated animals.

min after LTD₄ administration (Figure 4). ICI-192,605 and OKY-046 blocked the transient increase in mean systemic blood pressure induced by LTD₄, with a significant difference from 0.5 to 1 min until 4 min after instillation of LTD₄ ($P < 0.005$ and $P < 0.01$ at 2.5 min after LTD₄, respectively) (Figure 4). Instilled PGF_{2α} (20 nmol) and U-46619 (0.02 nmol) caused a significant fall in systemic mean blood pressure (Table 1). However, an instillation of saline also caused a small fall. ICI-192,605 abolished the PGF_{2α}-induced fall in blood pressure. In contrast, OKY-046 had no inhibitory effect on PGF_{2α}-induced changes in blood pressure.

TxB₂ concentration in BALF after instilled LTD₄

The recovery percentage of BALF was 27 ± 2 and $44 \pm 0.2\%$ in animals given either instilled LTD₄ or vehicle respectively. Instilled LTD₄ induced a significant increase in TxB₂ (14.6 ± 2.0 pM, $P < 0.01$) in bronchoalveolar lavage fluid obtained 1.5 min after challenge, compared to the animals given instilled vehicle (1.0 ± 0.1 pM).

Discussion

We have shown that both airflow obstruction and airway plasma exudation induced by LTD₄ instilled directly into the airways are significantly attenuated by both ICI-192,605, a thromboxane A₂ receptor (TP receptor) antagonist, and OKY-046, a thromboxane (Tx) synthetase inhibitor, in anaesthetized guinea-pigs. In addition, instilled LTD₄ resulted in a large increase of the level of TxB₂ in bronchoalveolar lavage fluid (BALF). ICI-192,605, but not OKY-046, also blocked both the biphasic increase in R_L and airway plasma

Figure 3 Extravasation of Evans Blue dye induced by instilled leukotriene D₄ (LTD₄, a), prostaglandin F_{2α} (PGF_{2α}, b), and U-46619 or saline (c) at different airway levels in untreated animals (thickly hatched columns), compared to animals given instilled saline (thinly hatched columns). Animals were also pretreated with either ICI-192,605 (0.5 mg kg⁻¹, i.v.; solid columns) or OKY-046 (30 mg kg⁻¹, i.v.; stippled columns). IPA = intrapulmonary airways. * $P < 0.05$; ** $P < 0.001$; *** $P < 0.005$ compared to untreated animals given instilled LTD₄ or PGF_{2α}. † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.005$ compared to animals given instilled saline. *n* = 4–9 as indicated at the foot of each column.

Figure 4 Effect of ICI-192,605 (●: 0.5 mg kg⁻¹, *n* = 7) and OKY-046 (▲: 30 mg kg⁻¹, *n* = 6) given i.v. 5 min before instillation of leukotriene D₄ (LTD₄, 0.2 nmol) on the time-course of the decrease in systemic blood pressure induced by LTD₄, compared with untreated animals given instilled LTD₄ (○: *n* = 9) or saline (□: 0.9% NaCl, *n* = 6). **P* < 0.05; ***P* < 0.005 compared to untreated animals.

exudation induced by PGF_{2α} instilled into the tracheal lumen. These findings suggest that LTD₄-induced airway plasma exudation and airflow obstruction may be mediated in part, via TxA₂ generation, but that the airway responses to PGF_{2α} may be mediated via direct activation of TP receptors, rather than via TxA₂ generation.

Bronchoconstriction produced by LTD₄ administered through the intratracheal route may be due to one or more of the following mechanism: (1) reflex stimulation via vagal pathways, (2) release of local mediators, such as cyclo-oxygenase products, (3) via direct effects (Smith *et al.*, 1987). In the present study, instillation of LTD₄ produced an immediate and subsequently persistent increase in R_L, with an immediate peak at 45 s. Neither ICI-192,605 nor OKY-046 significantly affected the initial increase in R_L, suggesting that the immediate airflow obstruction after LTD₄ may be mediated via cholinergic reflexes (Hirshman *et al.*, 1983) or due to a direct effect on airway smooth muscle (Smith *et al.*, 1987). Hamel *et al.* (1982) found that bronchoconstriction produced by aerosolized LTD₄ was not attenuated by cyclo-oxygenase inhibitors, although the effect of i.v. LTD₄ was. In the present study, the persistent increase in R_L following the immediate response after LTD₄ was significantly attenuated by both ICI-192,605 and OKY-046, strongly arguing for a role of TxA₂ in the airway responses to instilled LTD₄. Furthermore, we found in the present study that instilled LTD₄ resulted in a large increase in TxB₂, a stable TxA₂ metabolite, in BALF. Thus, our study strongly suggests that LTD₄-induced airflow obstruction in guinea-pigs may be mediated in part via TxA₂, in accord with the findings of Vincent *et al.* (1984) and Birch *et al.* (1988) who studied the effects of i.v. LTD₄. The discrepancy from the previous findings of Hamel *et al.* (1982) may be explained by different methods of administration and use of different inhibitors of the cyclo-oxygenase pathway (they used indomethacin). After intratracheal administration, it is possible that higher local concentrations of LTD₄ induce TxA₂ synthesis, compared with aerosolized LTD₄. Another possibility is that LTD₄ given as aerosol causes production of bronchodilator prostaglandins as well as TxA₂. Therefore, a reduction of both

the synthesis of TxA₂ and bronchodilator prostaglandins after indomethacin may explain the lack of attenuation of LTD₄-induced bronchoconstriction in the previous study (Hamel *et al.*, 1982). However, airway plasma exudation induced by aerosolized LTD₄ may well be attenuated by a cyclo-oxygenase inhibitor, because bronchodilator prostaglandins such as PGE₂ are known to enhance mediator-induced plasma exudation in the skin (Williams, 1979).

Woodward *et al.* (1983) found that indomethacin did not alter the increase in tracheal vascular permeability produced by intratracheal injection of LTD₄. By contrast, we found in the present study that both ICI-192,605 and OKY-046 significantly reduced instilled LTD₄-induced extravasation of dye in the trachea and proximal intrapulmonary airways, suggesting that LTD₄-induced airway plasma exudation may be mediated in part via TxA₂ generation. The discrepancy with the previous study of Woodward *et al.* (1983) may be explained by complete blockade of the TxA₂ receptors on the endothelial cells with ICI-192,605 and inhibition of TxA₂ synthesis with OKY-046, whereas the dose of indomethacin (5 mg kg⁻¹) used by Woodward *et al.* (1983) may have been insufficient to inhibit cyclo-oxygenase in the airways completely. In guinea-pigs, indomethacin may also have had the theoretical effect of shunting of arachidonic acid to the lipoxygenase pathway resulting in the synthesis of leukotrienes (Beasley *et al.*, 1989).

In the present study, instillation of PGF_{2α} into the trachea in guinea-pigs produced an immediate increase in R_L, peaking at approximately 15 s. The response to exogenously administered PGF_{2α} has been shown to be due in part, to cholinergic reflexes (Patel, 1975; Hadhazy *et al.*, 1982). We also found a delayed sustained increase in R_L, peaking at approximately 4 min. This biphasic response to PGF_{2α} was quite similar to that found with instilled U-46619 in the present study, although PGF_{2α} was about 1000 times less potent than U-46619 in producing a similar increase in R_L. As with instillation of U-46619, we have previously found that i.v. U-46619 produces an immediate and a delayed increase in R_L, both of which are abolished in animals pretreated with the TP receptor antagonist used in the present study, ICI-192,605 (Lötvall *et al.*, 1992). Furthermore, TP receptor antagonists block both PGF_{2α}-induced contraction in lung parenchymal strip in guinea-pigs *in vitro* (McKinniff *et al.*, 1991) and bronchoconstriction induced by intravenous administration of PGF_{2α} *in vivo* (Birch *et al.*, 1988). In the present study, we found that both the immediate and the delayed sustained increase in R_L after PGF_{2α} were abolished by pretreatment with ICI-192,605, but not with OKY-046. Therefore, it is unlikely that PGF_{2α} induces TxA₂ release from inflammatory cells to cause airflow obstruction, because a Tx synthetase inhibitor did not affect this response. The mechanism underlying the observed biphasic R_L response is not known, but our data provide evidence that PGF_{2α}-induced airflow obstruction may be mediated through activation of TP receptors. Alternatively, it is possible that ICI-192,605 may antagonize not only TP receptors but also other prostanoid receptor types such as DP, FP and EP₁ receptors (mediating the effects of PGD₂, PGF_{2α} and PGE₂, respectively; McKinniff *et al.*, 1991).

We have previously shown that i.v. U-46619 (2–200 nmol kg⁻¹) produces pronounced extravasation of Evans Blue dye at all airway levels, which is most prominent in intrapulmonary airways. In addition, this effect may be mediated via TP receptors, because extravasated dye was abolished in animals pretreated with the same dose of ICI-192,605 (Lötvall *et al.*, 1992) used in the present study. In the current study instillation of U-46619 (0.02 nmol) produced significant but rather small extravasation of dye at all airway levels. This may be explained by use of a lower dose of U-46619 or the different route of administration. Instilled PGF_{2α} produced a similar amount of extravasation of Evans Blue dye compared with U-46619, although the potency in inducing the effect was about 1000 times lower than with U-46619.

ICI-192,605 blocked the extravasation of Evans Blue dye induced by PGF_{2α}, suggesting that PGF_{2α}-induced airway plasma exudation is mediated through TP receptors. It is again considered unlikely that PGF_{2α} induces TxA₂ release from inflammatory cells to cause airway plasma exudation, since OKY-046 did not affect dye extravasation. Another possibility is that PGF_{2α} induces the release of other prostanoids, such as PGD₂ which activate TP receptors (McKenniff *et al.*, 1991).

We found that instillation of LTD₄ caused an immediate fall and subsequently a transient increase in mean systemic blood pressure approximately 1 min after administration. Both ICI-192,605 and OKY-046 blocked the transient increase in mean systemic blood pressure induced by LTD₄. This suggests that TxA₂ generation induced by instilled LTD₄ may be responsible for this effect, since TxA₂ is a potent vasoconstrictor (Lötvall *et al.*, 1992). Instilled U-46619 induced a significant fall in systemic blood pressure. Also, instilled PGF_{2α} caused a significant fall in systemic blood pressure, which was blocked with ICI-192,605, but not OKY-046, suggesting that blood pressure fall after PGF_{2α} may be mediated via TP receptor. It is likely that the effects of instilled U-46619 and PGF_{2α} on the blood pressure response is secondary to their airway effect. The transient partial recovery of blood pressure induced by instilled LTD₄ may be

explained by TxA₂ generation after an immediate peak airway response following the instillation.

In asthmatics, a recent double-blind, placebo-controlled trial of a TP receptor antagonist (GR32191) failed to demonstrate any decrease in airway responsiveness to acetylcholine (Stenton *et al.*, 1992). However, of particular interest to our present study is the finding that enhanced airway responsiveness to histamine following inhalation of LTE₄ in asthmatics is inhibited by prior ingestion of indomethacin (Christie *et al.*, 1992), implying that LTE₄-induced hyperresponsiveness in part is due to the release of TxA₂, or possibly some other cyclooxygenase product. In conclusion, our data suggest that airflow obstruction and airway plasma exudation induced by instilled PGF_{2α} may be mediated via activation of TP-receptors. Airway responses to instilled LTD₄ may in part, be produced by generation of TxA₂. However, from our present study, it is not possible to elucidate which cell is producing the thromboxane in response to exogenous leukotriene. Furthermore, the interactions between TxA₂, PGF_{2α} and LTD₄ in plasma exudation in human airways are not known, and deserve further investigation.

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