Br. J. Pharmac. (1984), **82**, 389–395

Studies on L-640,035: a novel antagonist of contractile prostanoids in the lung

R. Carrier, E.J. Cragoe[1], D. Ethier, A.W. Ford-Hutchinson, Y. Girard, R.A. Hall, P. Hamel, J. Rokach, N.N. Share[1], C.A. Stone[1] & P. Yusko

Merck Frosst Canada Inc., P.O. Box 1005, Pointe Claire-Dorval, Québec, Canada H9R 4P8

1 The effects of L-640,035 (3-hydroxymethyl-dibenzo [b,f] thiepin-5,5-dioxide) have been studied on pulmonary smooth muscle contraction *in vitro* and *in vivo*.

2 When studied *in vitro* on guinea-pig tracheal chains, L-640,035 produced significant shifts in the dose-response curves to a prostaglandin (PG) endoperoxide analogue (U-44069) (pA_2 7.0), $PGF_{2\alpha}$ (pA_2 5.9) and PGD_2 (pA_2 6.5). L-640,035 produced no significant shift in the dose-response curves to leukotriene D_4 or histamine and produced a small but statistically significant shift in the dose-response curve to 5-hydroxytryptamine (5-HT) (pA_2 5.2). With the exception of $PGF_{2\alpha}$, Schild analysis did not in general indicate competitive inhibition. The main metabolite of L-640,035, L-636,499, also produced significant parallel shifts in the dose-response curves to U-44069 (pA_2 6.0) and $PGF_{2\alpha}$ (pA_2 6.0), but with some reduction in the maximal contraction.

3 When L-640,035 was administered intravenously to guinea-pigs, significant inhibition of increases in pulmonary resistance or insufflation pressure induced by U-44069 (ED_{50} 0.16 mg kg^{-1}), leukotriene D_4 (ED_{50} 0.25 mg kg^{-1}) and 5-HT (ED_{50} 3.4 mg kg^{-1}) but not histamine (ED_{50} > 10 mg kg^{-1}) was observed.

4 When L-640,035 was administered intravenously to dogs a significant inhibition of increases in pulmonary resistance induced by U-44069 (ED_{50} 0.85 mg kg^{-1}) but not histamine (ED_{50} > 30 mg kg^{-1}) was observed.

5 When L-640,035 was administered by the intraduodenal route to dogs at doses of 3 and 10 mg kg^{-1} significant inhibition of increases in pulmonary resistance induced by sodium arachidonate (3 mg kg^{-1} i.v.) was observed with a duration of action of > 255 min.

6 It is concluded that L-640,035 is a novel, relatively selective, and orally active antagonist of the actions of contractile prostanoids on pulmonary smooth muscle.

Introduction

A number of investigations have suggested a role for cyclo-oxygenase products of arachidonic acid metabolism in pulmonary reactions such as those seen in human bronchial asthma (Piper & Vane, 1973; Hedqvist & Mathé, 1977; Austen & Orange, 1979). Firstly, prostaglandins and thromboxanes have been shown to be generated from antigen-challenged guinea-pig and human lung (Piper & Walker, 1973; Schulman *et al.*, 1981). Secondly human and guinea-pig lung have been shown to have both relaxant and contractile receptors for prostaglandins (Gardiner & Collier, 1980; Jones *et al.*, 1982). These findings have been confirmed *in vivo* where E prostaglandins have been shown to be bronchodilators and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) a bronchoconstrictor (Smith *et al.*, 1975; Mathé & Hedqvist, 1975). More recently other cyclo-oxygenase metabolites have been shown to be more potent bronchoconstrictors than $PGF_{2\alpha}$. These are thromboxane A_2 (TXA_2), the prostaglandin endoperoxides (PGG_2 and PGH_2) and PGD_2 (Svensson *et al.*, 1977; Hedqvist *et al.*, 1978; Wasserman *et al.*, 1980).

The present paper describes the properties of L-640,035 (3-hydroxymethyl-dibenzo [b,f] thiepin-5,5-dioxide) a novel antagonist of contractile prostanoids in pulmonary smooth muscle. The effects of L-636,499, the main metabolite of L-640,035 were also examined.

[1]Present address: Merck Sharpe and Dohme Research Laboratories, West Point, Pennsylvania, U.S.A.

Methods

In vitro *experiments: guinea-pig tracheal chain*

Male albino, Hartley strain guinea-pigs (300–500 g) were killed by a blow to the head and exsanguinated. Tracheae were removed, sectioned into rings of approximately 1 mm thickness and placed in physiological saline (0.9% w/v NaCl solution). Chains of 5 rings, constructed according to the method of Akcasu (1952) were mounted in 10 ml organ baths under 1 g resting tension in modified Krebs solution (NaCl 6.87, KCl 0.32, $MgSO_4$. $7H_2O$ 0.11, $CaCl_2$ 0.28, K_2HPO_4 0.16, $NaHCO_3$ 2.1; dextrose 2.1 g l^{-1}; equilibrated with 5% CO_2 in O_2). Indomethacin (1.4×10^{-1} M) was added to the Krebs solution to suppress endogenous prostaglandin synthesis and intrinsic tonus. Organ bath temperature was maintained at 37°C and 5% CO_2 in O_2 diffused continuously. Isometric tension changes were recorded from Grass FTO3 force displacement transducers connected to a Beckman type R dynograph. Following a 30 min equilibration period with regular organ bath solution changes the preparations were primed 2–3 times with the agonist under study. Following priming, an agonist control dose-response curve was obtained using a cumulative dose schedule (4–8 doses).The preparations were then washed at regular intervals until resting baseline tension had returned. After an appropriate rest period (1–1.5 h) the agonist dose-response curve was repeated in the presence of an antagonist drug concentration. For assay purposes L-640,035 was dissolved in 50% (v/v) aqueous polyethylene glycol (mol. wt. 200) and L-636,499 in water with the addition of stoichiometric amounts of $NaHCO_3$ and stirring. Drug doses were delivered in 100 µl volumes 5 min before the second agonist challenge. Total cumulative agonist doses did not exceed 100 µl per bath. EC_{50} values were obtained by regression analysis and used to calculate pA_2 values by the method of Tallarida & Murray (1981).

In vivo *studies*

U-44069 and histamine-induced bronchoconstriction in anaesthetized guinea-pig Male albino Hartley strain guinea-pigs (400–500 g) were anaesthetized with urethane (1.5 g kg^{-1} i.p.) given succinylcholine chloride (5 mg kg^{-1} s.c.) to suppress spontaneous respiration and artificially ventilated at 60 breaths min^{-1}, with volume adjusted to provide an end inspiratory pressure of 10 cmH_2O. Changes in airway resistance (R_L), dynamic compliance (C_{Dyn}) and tidal volume were monitored with an on-line pulmonary function computer (Buxco Electronics Inc., Sharon, Conn. U.S.A.). Tidal air flow was recorded from a Fleisch pheumotachograph (No. 0000) inserted between the tracheal cannula and respirator and connected to a Statham PM97 differential pressure transducer. Pulmonary pressure was obtained from a cannula placed between the tracheal cannula and the pheumotachograph and connected to one part of a Statham PM5 ± 0.7 differential pressure transducer. The transducers were connected to a pulmonary function computer with input to a Beckman type R dynograph which recorded the derived analogue data in $cmH_2O\ l^{-1}\ s^{-1}$ (R_L) and ml cmH_2O^{-1} (C_{Dyn}). Increases in R_L and decreases in C_{Dyn} were induced at 10 min intervals with U-44069 (2 µg kg^{-1} i.v.) or histamine (3 µg kg^{-1} i.v.) bolus injections into a cannulated jugular vein. The preparation was hyperinflated to 30 cmH_2O, 3 min before each challenge. Cumulative intravenous doses of L-640,035 dissolved in 50% (v/v) aqueous polyethylene glycol (mol. wt. 200) in a volume of 0.1 ml kg^{-1} were injected 2 min before each subsequent agonist challenge. Reduction of the agonist response was calculated as % of the control response preceding the initial drug injection. ED_{50}s (dose required to inhibit the induced increase in R_L by 50%) were calculated by regression analysis.

Leukotriene D_4-induced bronchoconstriction in guinea-pig Male albino Hartley strain guinea-pigs (350–400 g) were anaesthetized as above and artificially ventilated at 60 breaths min^{-1}. Changes in insufflation pressure (resistance to inflation) were measured using a Statham PM-5E differential pressure transducer recording on a Beckman type R dynograph, as described by Jones *et al.*, (1982). Increases in insufflation pressure (197 ± 19%: mean ± s.e.mean, $n = 13$) were induced with leukotriene D_4 (0.2 µg kg^{-1} i.v.) at 20 min intervals. Cumulative intravenous doses of L-640,035 dissolved in 50% (v/v aqueous polyethylene glycol, mol. wt. 200) in a volume of 0.1 ml kg^{-1} were injected 5 min before each subsequent agonist challenge. Reduction of the agonist response was calculated as % of the control response preceding the initial drug dose. ED_{50}s were calculated by regression analysis.

U-44069, histamine and arachidonic acid-induced bronchoconstriction in anaesthetized dog Mongrel dogs of either sex (4–12 kg) were anaesthetized with thiopentone (25 mg kg^{-1} i.v.) and a cuffed endotracheal tube inserted. Anaesthesia was maintained with sodium pentobarbitone intravenous infusion (0.55 mg $kg^{-1}\ min^{-1}$) into a cannulated femoral vein for approximately 30 min. The dose was then reduced to 0.1 mg $kg^{-1}\ min^{-1}$ for the remainder of the experiment. Spontaneous respiration was suppressed with (+)-tubocurarine (0.1 mg kg^{-1} i.m.) and the dog artificially respired at 20 breaths min^{-1} with volume adjusted to provide 8–9 cmH_2O inspiratory pres-

sure. Changes in airway resistance (R_L), dynamic compliance (C_{Dyn}) and tidal volume were monitored with an on-line Buxco pulmonary function computer. Gas flow was recorded from a Fleisch pneumotachograph (No. 0) inserted between the endotracheal tube and respirator and connected to the ports of a Statham P97 differential pressure transducer. Transpulmonary pressure was measured through a Statham PM5 ± 0.7 differential pressure transducer, with ports connected to an oesophageal balloon and to the endotracheal tube distal to the pheumotachograph. Both transducers were connected to the Buxco pulmonary function computer which provided an analogue record of R_L and C_{Dyn} on a Beckman type R dynograph, in $cmH_2O\ l^{-1}\ s^{-1}$ and ml cmH_2O^{-1}, respectively. Changes in R_L (increase) and C_{Dyn} (decrease) were induced with either U-44069 (3 $\mu g\ kg^{-1}$ i.v.), histamine (20 $\mu g\ kg^{-1}$ i.v.) or sodium arachidonate (3 $mg\ kg^{-1}$ i.v.) bolus injections into a cannulated femoral vein at 30 min intervals. Fifteen min before each challenge the lungs were hyperinflated to 24–30 cmH_2O (3 breaths). Agonist challenges were repeated until the response (increase in R_L) had stabilized. Mean (± s.e.mean) % control changes in R_L and C_{Dyn} respectively, obtained with the three agonists, were 87 ± 9 and 40 ± 3, $n = 11$ (U-44069), 53 ± 1 and 38 ± 2, $n = 9$ (histamine) and 63 ± 13 and 37 ± 2, $n = 12$ (sodium arachidonate). L-640,035 (dissolved in 50% v/v aqueous polyethylene glycol, mol. wt. 200) was administered in cumulative intravenous doses 5 min before consecutive agonist challenges to determine the acute inhibitory ED_{50}. Single doses, administered intraduodenally, through a ventral midline incision were used to determine absorption and duration of action. Inhibition was calculated as % of the last pre-drug agonist response, and ED_{50}s calculated by regression analysis.

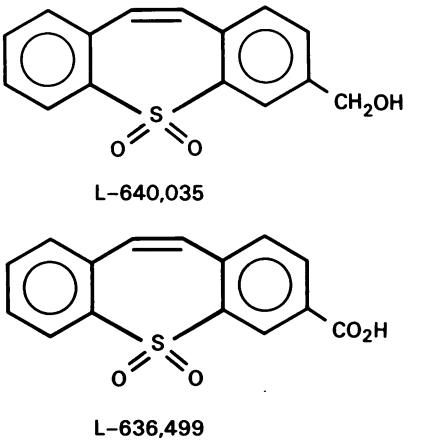

Figure 1 Chemical structure of L-640,035 and its main metabolite L-636,499

Drugs

Drugs used were L-640,035 (3-hydroxymethyl-dibenzo [b,f] thiepin 5,5-dioxide), L-636,499 (dibenzo [b,f] thiepin-3-carboxylic acid, 5,5-dioxide), indomethacin, leukotriene D_4 (Merck Frosst), urethane (Koch Light), succinylcholine chloride (Abbott), U-44069 (15 (S)-hydroxy-9α, 11α-(epoxymethano)-prosta-5-*cis*, 13 *trans*, dienoic acid) (Upjohn), 5-hydroxytryptamine creatinine sulphate (5-HT) (+)-tubocararine (Calbiochem), $PGF_{2\alpha}$, PGE_2 (ONO) PGD_2 (Salford Fine Chemicals), thiopentone (Abbot), sodium pentobarbitone (Abbott), histamine and arachidonic acid (Sigma). Unpublished results have shown that L-640,035 is rapidly converted *in vivo*, but not *in vitro* in tissue baths, to L-636,499. The structure of both compounds is shown in Figure 1.

Table 1 Effects of L-640,035 on agonist-induced contractions of non-tonal guinea-pig tracheal smooth muscle *in vitro*

Agonist	pA_2	m	r	n
U-44069	7.0	−0.56	−0.8	26
	(6.4–7.6)	(−0.37, −0.75)		
$PGF_{2\alpha}$	5.9	−0.94	−0.81	17
	(5.3–6.5)	(−0.58, −1.3)		
PGD_2	6.5	−0.53	−0.53	17
	(5.1–7.8)	(−0.19, −0.87)		
5-HT	5.2	−1.45	−0.92	18
	(5.0–5.4)	(−1.13, −1.77)		
Histamine	NS (1.2×10^{-4}M)			6
Leukotriene D_4	NS (2.7×10^{-5}M)			6

The pA_2 values and the slopes of the lines (m) are shown with 19/20 confidence limits in parentheses, together with the regression correlation coefficient (r) and the number of experiments (n). NS denotes no significant shift on the agonist dose-response curve in the presence of the antagonist concentration, shown in parentheses.

Results

In vitro *studies using guinea-pig tracheal chains*

L-640,35 was tested as an antagonist of the contractions induced by the stable endoperoxide analogue, U-44069, and other contractile agonists; results are shown in Table 1. The compound inhibited the contractile responses to U-44069, $PGF_{2\alpha}$ PGD_2 and 5-HT, with pA_2 values of 7.0, 5.9, 6.5 and 5.2, respectively. No significant shifts of the dose-response curves to histamine or LTD_4 were obtained at the relatively high drug concentrations of 1.2×10^{-4} M and 2.7×10^{-5}M, respectively, These results indicate that L-640,035 is a relatively potent and selective antagonist of prostanoid receptor mediated tracheal smooth muscle contractions in the guinea-pig (*in vitro*) with some activity against 5-HT-induced contraction. Comparison of the slopes from the Schild regression data (Table 1) suggests that the compound is a competitive antagonist of $PGF_{2\alpha}$ ($m = -0.94$) but not U-44069 or PGD_2. However, at concentrations from 3.7×10^{-6} to 1×10^{-4}M L-640,035 caused moderate but statistically significant reductions in the maximal contraction when used with the prostanoid agonists and 5-HT, but not with histamine or leukotriene D_4. Since L-636,499, a 3-carboxylic acid analogue of L-640,035 was shown to be the primary metabolite of the latter compound *in vivo* in dog and rat, it was tested *in vitro* in a similar manner to L-640,035. Results are shown in Table 2. In these tests L-636,499 was equipotent against both U-44096 and $PGF_{2\alpha}$-induced contractions with pA_2 values of 6.0. The slopes of the Schild regression for both compounds were not significantly different from -1, suggesting competitive inhibition. Moderate (16–26%), but significant reductions in the maximal contractions, however, were recorded at drug concentrations from 1×10^{-5} to 3.5×10^{-4} M. No inhibition of the histamine response was seen at a high drug concentration (3.5×10^{-4} M), while the pA_2 value obtained against 5-HT (4.3) was approximately 2 orders of magnitude lower than those found for U-44069 and $PGF_{2\alpha}$. Experiments with the appropriate vehicle controls produced no significant shifts in the dose-response curves of any of the agonists tested. Low doses of indomethacin were used in all *in vitro* experiments to suppress endogenous synthesis of prostaglandins. At these doses incomplete inhibition of prostaglandin synthesis and suppression by the antagonists of the effects of prostaglandins released during the experiment, may explain the observed reduction in the maximal contractions.

In other experiments PGE_2 (4 ng ml^{-1}) was used to induce consistent sub-maximal contractions of indomethacin (0.5 μg ml^{-1}) treated guinea-pig tracheal chains. Increasing concentrations of L-640,035 or L-636,499 were added to the organ bath 5 min before agonist challenge. Reduction of the PGE_2 response was calculated as % of the standard PGE_2 contraction. In these experiments EC_{50}s of approximately 2.0 and 1.0 μg ml^{-1} were obtained for L-640,035 and L-636,499, respectively. Neither compound at concentrations up to 3.5×10^{-5} M inhibited the PGF_2 (2 μg ml^{-1})-induced relaxation of guinea-pig tracheal chains in which tonus had been induced with carbachol (100 ng ml^{-1}).

Inhibition of bronchoconstriction in vivo

The relative selectivity and *in vivo* antagonist potency of L-640,035 in anaesthetized guinea-pigs and dogs is shown in Table 3. In these tests, intravenous ED_{50}s of 0.16 ± 0.03 mg kg^{-1} and 0.84 ± 0.09 mg kg^{-1} (means ± s.e.mean) were obtained for L-640,035 against U-44069-induced increases in R_L in the guinea-pig and dog, respectively. No significant inhibition of histamine-induced increases in airway resistance was recorded at doses of 10 and 30 mg kg^{-1} (i.v.) in the guinea-pig or dog respectively. Inhibition of the compliance response (reduction) following U-44069 challenge was obtained in both species, but at slightly higher doses than those needed to inhibit the resistance parameter (R_L). The compound was ineffective against the histamine-induced changes in C_{Dyn} in both species. Some inhibition of

Table 2 Effect of L-636,499 on agonist-induced contractions of non-tonal guinea-pig tracheal smooth muscle *in vitro*

Agonist	*pA2*	m	r	n
U-44069	6.0 (5.6–6.3)	−0.97 (−0.7, −1.24)	−0.85	24
$PGF_{2\alpha}$	6.0 (5.7–6.2)	−0.96 (−0.81, −1.11)	−0.94	24
5-HT	4.3 (apparent)			6
Histamine	NS (3.5×10^{-4}M)			6

Legend as for Table 1. (apparent) Signifies a pA_2 value determined for a single antagonist concentration.

Table 3 Inhibition of agonist-induced bronchoconstriction *in vivo* by L-640,035 administered intravenously

Agonist	*Dose* ($\mu g\,kg^{-1}$ i.v.)	*Species*	*ED_{50} (R_L)* ($mg\,kg^{-1}$)	*ED_{50} (C_{Dyn})* ($mg\,kg^{-1}$)	n
U-44069	2.0	Guinea-pig	0.16±0.03	0.1–0.3	5
Histamine	3.0	Guinea-pig	>10.0	>10.0	5
5-HT	20.0	Guinea-pig	3.4±0.5	3.7±1.6	4
Leukotriene D_4	0.2	Guinea-pig	0.25±0.04*		7
U-44069	3.0	Dog	0.84±0.09	1.0–3.0	5
Histamine	20.0	Dog	>30.0	>30.0	4

ED_{50}s (means ±s.e.mean) are the doses required to inhibit by 50%, the induced increases in pulmonary resistance (R_L) and decrease in dynamic compliance (C_{Dyn}) except where indicated (*) when the ED_{50} refers to the dose required to similarly inhibit insufflation pressure.

5-HT-induced bronchoconstriction was observed although the ED_{50} was approximately 20 times higher than that for inhibition of bronchoconstriction induced by U-44069. It has been shown that the increase in insufflation pressure in anaesthetized guinea-pigs, induced by leukotriene D_4 (0.2 $\mu g\,kg^{-1}$ i.v.) is mediated indirectly through the generation of thromboxane A_2. This response can be inhibited by both cyclo-oxygenase and thromboxane synthetase inhibitors (Hamel *et al.*, 1982). L-640,035 was a potent inhibitor of leukotriene D_4-induced bronchoconstriction in this species with an ED_{50} of 0.25 ± 0.04 $mg\,kg^{-1}$ (i.v.) (mean ± s.e.mean).

Increases in R_L and reductions in C_{Dyn} were induced with bolus i.v. injections of sodium arachidonate (3 $mg\,kg^{-1}$) in anaesthetized dogs. Single doses of L-640,035 (3 or 10 $mg\,kg^{-1}$) were administered intraduodenally and the development and duration of the induced bronchoconstriction (increase in R_L) recorded. Results are shown in Figure 2. Both doses of the compound inhibited the induced increase in airway resistance to a similar extent (56 ± 4%). Maximum inhibition was observed 75 min after drug administration, with a duration ($t_{\frac{1}{4}}$) in excess of 4 h.

Discussion

Thromboxane A_2 (TXA_2) mediates a number of biological activities including the aggregation of platelets and contraction of respiratory and vascular smooth muscle (Svensson *et al.*, 1976; 1977). In general, TXA_2 is more potent than either PGH_2, PGG_2 or $PGF_{2\alpha}$ (Svensson *et al.*, 1977) and it is generally assumed that all these compounds interact with a single thromboxane receptor site. Due to the instability of TXA_2, stable thromboxane or prostaglandin endoperoxide analogues have been used. In the present studies we have used a derivative of PGH_2 in which the 9–11 peroxide bridge has been replaced by a 9,11 epoxymethano bridge (U-44069) (Bundy, 1975). This compound has been shown to

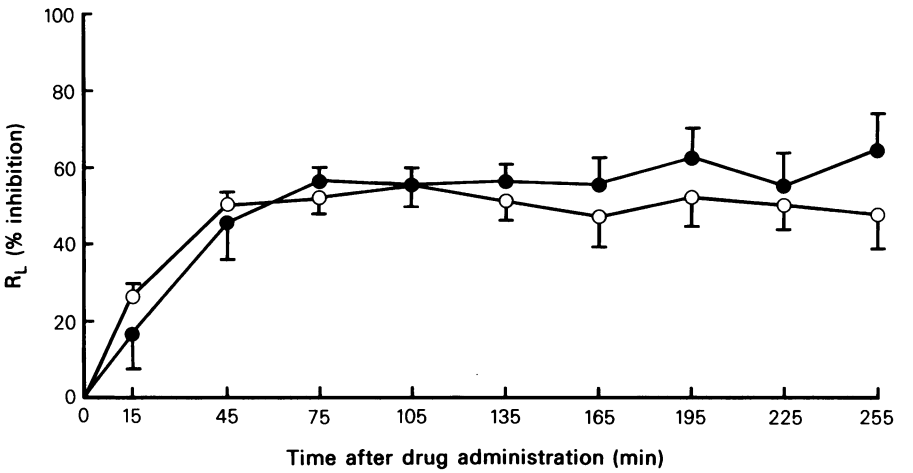

Figure 2 Inhibition of arachidonic acid (3 $mg\,kg^{-1}$ i.v.)-induced increases in airway resistance (R_L) following intraduodenal administration of L-640,035, 3 mg kg^{-1} (●) and 10 mg kg^{-1} (○), in anaesthetized dogs.

have similar actions to prostaglandin endoperoxides and thromboxanes on platelets (Malmsten, 1976; MacIntyre *et al.*, 1978) and interacts as a partial agonist with the thromboxane receptor on guinea-pig trachea, the dog saphenous vein and rabbit aorta (Jones *et al.*, 1982b).

The guinea-pig trachea has both relaxant and contractile receptor sites for prostaglandins (Gardiner & Collier, 1980; Jones *et al.*, 1982a). On the basis of studies with the thromboxane antagonist EP 045, Jones *et al.*, (1982b) postulated that there were two contractile receptors on the guinea-pig trachea for prostaglandins. The first was activated by TXA_2, prostaglandin endoperoxides and endoperoxide and thromboxane analogues and was blocked by EP 045. The second contractile receptor site was activated by PGE_2 and PGF_2 analogues and was not blocked by EP 045. EP 045 produced partial blockade of both PGD_2 and $PGF_{2\alpha}$-induced contractions and the authors suggested that these prostaglandins interacted with both contractile receptor sites. The spectrum of activity of L-640-035 differs from that of EP 045 in that it antagonizes contractions induced by both U-44069 and PGE_2 as well as $PGF_{2\alpha}$ and PGD_2. This suggests that if two separate contractile receptors exist then L-640,035 can interact with both and hence may have a broader range of antagonist activity than compounds such as EP 045 which are chemically related in structure to TXA_2 (Jones *et al.*, 1982b). It is difficult to ascertain whether L-640,035 is a competitive antagonist on the guinea-pig tracheal chain as Schild plots with slopes close to -1 were only obtained against $PGF_{2\alpha}$. Some reductions in maximal contractions were observed at the higher antagonist concentrations. It is, however, clear from Table 1 that the antagonist shows selectivity for contractile prostanoids when compared to other agonists, although some activity was observed against contractions induced by 5-HT. L-640,035 is metabolized *in vivo* to another active compound, L-636,499, and data shown in Table 2 indicate that the metabolite has qualitatively similar actions to the parent compound.

The ability of L-640,035 to block tracheal smooth muscle contractions induced by contractile prostanoids *in vitro* was also demonstrated *in vivo*. When administered intravenously, L-640,035 was a potent inhibitor of broncho-constriction induced by U-44069 but not histamine in the guinea-pig and dog. It is of particular interest that L-640,035 will inhibit the contractile response of guinea-pig tracheal smooth muscle to PGD_2, as PGD_2 has been shown to be the major cyclo-oxygenase product released by human lung mast cells, (Lewis *et al.*, 1982). It seems probable that PGD_2, together with histamine, is a major mediator of the episodic attacks observed in patients with systemic mastocytosis (Roberts *et al.*, 1980).

The antagonist also blocked bronchoconstriction induced by a low i.v. dose of leukotriene D_4 in the guinea-pig. Previous results have shown that this response is also blocked by cyclo-oxygenase and thromboxane synthetase inhibitors (Hamel *et al.*, 1982) demonstrating that bronchoconstriction in this model is due to the generation of TXA_2. The present results confirm these conclusions and provide *in vivo* evidence that L-640,035 blocks the actions of TXA_2 as well as those of endoperoxide analogues.

Rapid infusion of arachidonic acid into dogs results in bronchoconstriction associated with decreases in airway diameters (Spannhake *et al.*, 1978; Quan *et al.*, 1982). The response is thought to be due to the generation of bronchoconstrictor metabolites of arachidonic acid (PGG_2, PGH_2, TXA_2, $PGF_{2\alpha}$) since it is blocked by indomethacin. In the present work, the airway response to arachidonic acid in the dog, was substantially blocked by L-640,035, administered intraduodenally, at doses of 3 and 10 mg kg^{-1} (Figure 1), supporting the conclusions of Spannhake *et al.* (1982). The results indicate that L-640,035 is well absorbed from the gastro-intestinal tract of the dog, and with a recovery time ($t_{\frac{1}{2}}$) in excess of 255 min, has a reasonable duration of action.

Despite evidence that prostaglandins or other cyclo-oxygenase products of arachidonic acid may play a role in the pathophysiology of some pulmonary diseases such as asthma, the effects of administering cyclo-oxygenase inhibitors to asthmatic patients have been equivocal. Thus indomethacin was reported to be ineffective (Smith & Dunlop, 1975; Fish *et al.*, 1981), while in a small number of patients aspirin precipitated severe asthmatic attacks (Szczeknic & Gryglewski, 1978; Szczeklic *et al.*, 1980). In these patients the effect is believed to be due to the loss of endogenous bronchodilator E prostaglandins, with a subsequent increase in histamine release and the possible diversion of arachidonic acid to the 5-lipoxygenase metabolic pathway. L-640,035 is a selective antagonist of the contractile effects of prostaglandins and thromboxane A_2 on the lung, and should not restrict the endogenous generation of the relaxant PGE_2 or inhibitory PGI_2, or increase the availability of arachidonic acid to the lipoxygenase metabolic pathway. The compound may thus have advantages over the cyclo-oxygenase inhibitors, both as a potential therapeutic agent and tool, in the elucidation of the role of the cyclo-oxygenase products of arachidonic acid metabolism in asthma. The effects of L-640,035 and L-636-499 on platelet function and contraction of vascular and uterine smooth muscle are being studied.

References

AKCASO, A. (1959). The physiological and pharmacologic characteristics of the tracheal muscle. *Arch. int. Pharmacodyn.*, **122**, 201–207.

AUSTEN, K.F. & ORANGE, R.P. (1979). Bronchial asthma: The possible role of the chemical mediators of immediate hypersensitivity in the pathogenesis of subacute chronic disease. *Am. Rev. Respir. Dis.*, **112**, 423–34.

BUNDY, G.L. (1975). Synthesis of prostaglandin endoperoxide analogs. *Tetrahedron Lett.*, **24**, 1957–1960.

FISH, B.J., ANKIN, M.G., ATKINSON Jr., N.F. & PETERMAN, V.I., (1981). Indomethacin modification of immediate-type immunologic airway responses in allergic asthmatic and non-asthmatic subjects. *Am. Rev. Respir. Dis.*, **123**, 609–614.

GARDINER, P.J. & COLLIER, H.O.J. (1980) Specific receptors for prostaglandins in airways. *Prostaglandins*, **6**, 819–841.

HAMEL, R., MASSON, P., FORD-HUTCHINSON, A.W., JONES, T.R., BRUNET, G. & PIECHUTA, H. (1982). Differing mechanisms for leukotriene D4-induced bronchoconstriction in guinea pigs following intravenous and aerosol administration. *Prostaglandins*, **24**, 419–432.

HEDQVIST, P. & MATHE, A.A. (1977). Lung function and the role of prostaglandins. In *Asthma physiology, immunopharmacology and treatment*, ed. Lichtenstein L.M. & Austen, K.F. pp. 131–146. 2nd ed. New York: Academic Press.

HEDQVIST, P. STRANDBERG, K. & HAMBERG, M. (1978). Bronchial and cardiovascular actions of prostaglandin endoperoxides and an endoperoxide analogue. *Acta physiol. scand.*, **103**, 299–307.

JONES T., MASSON, P., HAMEL, R., BRUNET, G., HOLME, G., GIRARD, Y., LARUE, M. & ROKACH, J. (1982a). Biological activity of leukotriene sulfones on respiratory tissues. *Prostaglandins*, **24**, 279–291.

JONES, R.L., PEESAPATI, V. & WILSON, N.H. (1982b). Antagonism of the thromboxane-sensitive contractile systems of the rabbit aorta, dog saphenous vein and guinea-pig trachea. *Br. J. Pharmac.*, **76**, 423–438.

LEWIS, R.A., SOTER, N.A., DIAMOND, P.T., AUSTEN, K.F., OATES, J.A. & ROBERTS, L.J. (1982). Prostaglandin D_2 generation after activation of rat and human mast cells with anti-IGE. *J. Immunol.*, **129**, 1627–1631.

MACINTYRE, D.E., SALZMAN, E.W. & GORDON, J.L. (1978). Prostaglandin receptors in human platelets. Structure-activity relationships of stimulatory prostaglandins. *Biochem. J.*, **174**, 921–929.

MALMSTEN, C. (1976). Some biological effects of prostaglandin endoperoxide analogs. *Life Sci.*, **18**, 169–176.

MATHE, A.A. & HEDQVIST, P. (1975). Effects of prostaglandins $F_{2\alpha}$ and E_2 on airway conductance in healthy subjects and asthmatic patients. *Am. Rev. Respir. Dis.*, **111**, 313–320.

PIPER, P.J. & VANE, J. (1971). The release of prostaglandins from lung and other tissues. *Ann. New York Acad. Sci.*, **180**, 363–385.

PIPER, P.J. & WALKER, J.C. (1973). The release of spasmodic substances from human chopped lung tissue and its inhibition. *Br. J. Pharmacol.*, **47**, 291–304.

QUAN, S.F., MOON, M.A. & LEMON, R.J. (1982). Effects of arachidonic acid, $PGF_{2\alpha}$, and a PGH_2 analogue on airway diameters in dogs. *J. appl. Physiol.*, **53**, 1005–1014.

ROBERTS, L.J., SWEETMAN, B.J., LEWIS, R.A., AUSTEN, K.F. & OATES, J.A. (1980). Increased production of prostaglandin D_2 in patients with systemic mastocytosis. *New Engl. J. Med.*, **303**, 1400–1404.

SCHULMAN, E.S., NEWBALL, H.H., DEMERS, L.M., FITZPATRICK, F.A. & ADKINSON, N.F. (1981). Anaphylactic release of thromboxane A_2, prostaglandin D_2, and prostacyclin from human lung parenchyma. *Am. Rev. Respir. Dis.*, **124**, 402–406.

SMITH, A.P., CUTHBERT, M.F. & DUNLOP, L.S. (1975). Effects of inhaled prostaglandins E_1, E_2, and $F_{2\alpha}$ on the airway resistance of healthy and asthmatic man. *Clin. Sci. Mol. Med.*. **48**, 421–430.

SMITH, A.P. & DUNLOP, L. (1975). Prostaglandins and asthma. *Lancet*, **1**, 39.

SPANNHAKE, E.W., LEMEN, R.J., WEGMANN, M.J., HYMAN, A.L. & KADOWITZ, P.J. (1978). Effects of arachidonic acid and prostaglandins on lung function in the intact dog. *J. appl. Physiol.*, **44**, 397–405.

SVENSSON, J., HAMBERG, M. & SAMUELSSON, B. (1976). On the formation and effects of thromboxane A_2 in human platelets. *Acta physiol. scand.*, **98**, 285-294.

SVENSSEN, J., STRANDBERG, K., TUVEMO, T. & HAMBERG, M. (1977).Thromboxane A_2: Effects on airway and vascular smooth muscle. *Prostaglandins*, **14**, 425–36

SZCZEKLIK, A. & GRYGLEWSKI, R.J. (1978). Prostaglandins and aspirin-sensitive asthma. *Am. Rev. Respir. Dis.*, **118**, 799–800.

SZCZEKLIK, A., GRYGLEWSKI, R.J., CZERNIAWSKA-MYSIK, G. & NIZANKOWSKA, E. (1980). Aspirin, prostaglandins and bronchial asthma. *Advances in Prostaglandin and Thomboxane Research*, **7**, 993–994.

TALLARIDA, R.J. & MURRAY, R.B. (1981). *Manual of pharmacological calculations.* New York: Springer-Verlag.

WASSERMAN, M.A., GRIFFIN, R.L. & MARSALISI, F.B. (1980). Potent bronchoconstrictor effects of aerosolized prostaglandin D_2 in dogs. *Prostaglandins*, **20**, 703–715.

(Received July 29, 1983. Revised January 9, 1984).