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Prevention and reversal of endotoxin-induced pulmonary hypertension by a leukotriene antagonist

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Prevention and reversal of endotoxin-induced pulmonary hypertension by a leukotriene antagonist. T. Ahmed, B. Weichman, M.A. Wasserman, R. Muccitelli, S. Tucker, B. Marchette.

ABSTRACT: We investigated the role of leukotrienes in endotoxin-induced changes in pulmonary circulation. In six conscious sheep, haemodynamic measurements were obtained for the calculation of pulmonary vascular resistance (PVR), along with measurements of arterial oxygen tension (Pa_{O_2}), leucocyte count (WBC), thromboxane B_2 (TxB_2), 6-Keto-$PgF_{1\alpha}$ and $PgF_{2\alpha}$, before and at predetermined intervals after a 10-min infusion of E. coli endotoxin (0.3 μg/kg), with and without treatment with the leukotriene receptor antagonist, FPL-57231. Endotoxin caused a biphasic response (*i.e.*, phase I = 0–1 h, phase II = 1.5–4 h), with a mean ± SE increase in PVR to 415 ± 112% of baseline during phase I and a lesser increase of 175% (range = 153–199%) of baseline during phase II. Mean ± SE Pa_{O_2} decreased from 86 ± 4 to 67 ± 6 mmHg and WBC count decreased from 8.6 ± 0.6 to 2.8 ± 0.7 thousand/mm^3 during phase I, whereas TxB_2 increased from 145 ± 28 to 3164 ± 1082 pg/ml, 6-Keto-$PgF_{1\alpha}$ from 129 ± 14 to 438 ± 114 pg/ml and $PgF_{2\alpha}$ from 122 ± 7 to 242 ± 43 pg/ml. One hour infusion of FPL-57231 (1 mg/kg/min) administered prior to and throughout phase I attenuated the phase I increases in PVR without preventing the increases in TxB_2; however, it partly attenuated 6-Keto-$PgF_{1\alpha}$ and enhanced generation of $PgF_{2\alpha}$ during phase I. Discontinuation of FPL-57231 was followed by an exaggerated response of PVR during phase II to an average of 209% of baseline (range = 186–235%). Four hours post-endotoxin, 10 min infusion of FPL-57231 (1 mg/kg/min) effectively reversed the increased PVR to basal levels. FPL-57231 had no effect on endotoxin-induced decreases in WBC count. We conclude that endotoxin-induced pulmonary hypertension is effectively prevented and reversed by a leukotriene antagonist.

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Endotoxaemia causes marked changes in the pulmonary circulation, which involve complex pathophysiological mechanisms. It has been shown that endotoxaemia results in a biphasic pulmonary vascular response in sheep [5–7, 28, 33]. The early phase (phase I) is characterized by marked pulmonary hypertension without alteration in microvascular permeability; whereas the second more prolonged phase (phase II) is characterized by moderately elevated pulmonary vascular resistance and abnormal microvascular permeability. Cyclooxygenase products may be involved in the pulmonary hypertensive response during phase I, as inhibitors of cyclooxygenase pathway like indomethacin, ibuprofen and meclofenamate have been observed to attenuate the response [5, 10, 28, 33]. Furthermore, increased levels of 6-Keto-$PgF_{1\alpha}$ (stable metabolites of prostacyclin) and thromboxane-B_2 (TxB_2, a stable metabolite of thromboxane A_2), have been measured in the blood and lymph during endotoxaemia [5, 10, 28, 33]. Cyclooxygenase inhibitors have also been shown to inhibit the endotoxin-induced increases in TxB_2 and 6-Keto-$PgF_{1\alpha}$ without blocking the late effects of endotoxin on pulmonary vascular resistance, microvascular permeability or the endotoxin-induced leucopenia [5, 10, 28, 33]. It is not known whether the cyclooxygenase inhibitors had any effect on the generation of 5-lipoxygenase products. Although increased levels of 5-hydroxyeicosatetraenoic acid (5-HETE) and 12-HETE have been observed in the lymph of sheep during endotoxaemia [27, 29], the role of leukotrienes in the pathophysiology of this syndrome remains controversial [5, 34]. Thus, the purpose of the present investigation was to study whether endotoxin-induced pulmonary hypertension could be prevented and reversed by FPL-57231, an antagonist of leukotrienes.

Materials and methods

A. Animal preparation

Six conscious adult ewes (weighing 24–33 kg) were used in these experiments. The sheep were restrained in the prone position and their heads were immobilized with a sling. The skin overlying the external jugular vein was anaesthetized with 4% lidocaine

solution, the jugular vein was exposed, and a 7F Swan-Ganz flow-directed thermodilution catheter was inserted into the vein and advanced until the tip lay within the pulmonary artery. All sheep had been previously prepared with a chronic carotid artery loop to facilitate arterial blood gas sampling and to measure systemic arterial pressure (Psa), using an indwelling 19-gauge needle. A slow infusion of heparinized physiologic saline solution was used to maintain the patency of the Swan-Ganz catheter and arterial needle. Each sheep received less than 300ml of this heparinized physiologic saline solution and the 5% glucose solution used for the thermodilution cardiac output measurements during the experiments. An intravenous catheter was placed in a leg vein for administration of FPL-57231.

B. Experimental techniques

1. Haemodynamic measurements: These have been described in detail previously [1–5]. Briefly, the Swan-Ganz catheter and the arterial needle were connected to strain gauges (Model P-23; Statham Instruments, Hato Rey, PR), which were referred to the mid-chest level. The pressures were recorded on an oscilloscope recorder (Model DR-12; Electronics for Medicine, White Plains, NY). After the measurements of mean pulmonary artery pressure (Ppa), the balloon at the tip of the Swan-Ganz catheter was inflated with 0.5–1.0 ml of air and the catheter advanced until a pulmonary arterial wedge pressure (Ppaw) reading was obtained. Immediately after measurement of Ppaw, the balloon at the catheter tip was deflated and the catheter pulled back to its original position.

With the Swan-Ganz catheter tip positioned in the pulmonary artery, a bolus of 10 ml of 5% glucose solution (1°C) was then injected into the proximal line of the Swan-Ganz catheter. Pulmonary blood flow (Q'p) was estimated by a thermodilution cardiac output computer (Model 9500; Edwards Laboratories, Santa Anna, CA) and averaged from 4–5 consecutive measurements [17]. Pulmonary vascular resistance (PVR) was defined as (Ppa − Ppaw)/Q'p, while systemic vascular resistance (SVR) as mean Psa/Q'p.

2. Arterial blood gas composition: Blood (2ml) was withdrawn in a heparinized syringe from the carotid loop. The blood samples were analysed at 37°C for pH, oxygen tension (Pa_{O_2}), and carbon dioxide tension (Pa_{CO_2}) with a radiometer (Model ABL-1: Radiometer, Copenhagen, Denmark).

3. Prostanoid assay: Arterial blood (10ml) was withdrawn in a heparinized syringe and stored in a vacutainer tube containing indomethacin (10^{-5} M). The blood samples were then centrifuged, and the plasma was collected and stored at −80°C until the day of assay. The plasma was utilized unextracted for the determination of TxB_2, 6-Keto-$PgF_{1\alpha}$ and $PgF_{2\alpha}$ concentrations by radioimmunoassay, using kits purchased from New England Nuclear, Boston, MA (TxB_2 and 6-Keto-$PgF_{1\alpha}$) and Seragen, Inc., Boston, MA ($PgF_{2\alpha}$) [18].

4. White blood cell count: Arterial white blood cell (WBC) count was performed manually using a haemocytometer.

C. Experimental protocol

Each sheep was studied on two different days at least one week apart. On day 1 the sheep were given endotoxin alone and on day 2 modification of endotoxin effects by FPL-57231 was studied. Thus, each animal served as its own control.

1. Pulmonary effects of a sublethal injection of endotoxin: Baseline measurements of Ppa, Ppaw, Psa, Q'p, and arterial blood gas composition were obtained after the animals had rested for 30 min. Blood samples were obtained for analysis of cyclooxygenase products and WBC count. *E. coli* endotoxin (0.3 μg/kg) was dissolved in 15 ml of physiological saline and infused over 10 min into the proximal Swan-Ganz line, via a Harvard infusion pump. Measurements of pulmonary haemodynamics, and arterial blood gases were obtained 5 min after endotoxin, every 15 min for the first hour, and then every 30 min up to 4 h. Blood samples for cyclooxygenase products and WBC count were obtained 30 min after the endotoxin and then every hour up to 4 h.

2. Modification of endotoxin effects by leukotriene antagonist (FPL-57231): Baseline haemodynamics were measured, and arterial blood was obtained for analysis of gas composition, cyclooxygenase products and WBC count. FPL-57231 (1 mg/kg/min) was dissolved in bacteriostatic injection water (concentration 1–1.5%), and administered into a peripheral leg vein as a continuous infusion via a Harvard infusion pump. This dose of FPL-57231, in sheep, has been previously shown to inhibit the pulmonary arterial pressor response to LTD_4 without attenuating the pressor effects of histamine and $PgF_{2\alpha}$ [1, 3]. FPL-57231 infusion was started 3 min before the endotoxin administration and infused continuously at the above mentioned rate for one hour. Endotoxin (0.3 ug/kg) was administered 3 min after the beginning of FPL-57231 infusion and measurements were repeated at predetermined intervals as in protocol 1. FPL-57231 was discontinued at the end of phase I (one hour). Four hours post-endotoxin, when all measurements had been obtained, FPL-57231 was reinfused (1 mg/kg/min) for 10 min to see if the pulmonary vascular changes are reversible.

Statistics

A two-way analysis of variance with repeated measures on both factors was used to evaluate changes over time as well as for comparison of data on different experiment days. When significant differences were found, a Newman-Keuls test was used to identify pairs that were different [23].

Results

A. Effects of endotoxin

Endotoxin caused a biphasic response with a peak increase in mean (±SE) PVR to 415±112% of baseline, respectively, during phase I (0–1 h) (fig. 1, table I). The peak increase in mean PVR was observed between 45–60 min after the endotoxin administration. During phase II (1.5–4 h), PVR remained elevated at an average of 175% (range

Table I. - Modification of endotoxin-induced changes in pulmonary haemodynamics and arterial blood gases by leukotriene antagonist FPL–57231

	Baseline	15 min	30 min	45 min	1 hr	1.5 hr	2 hr	3 hr	4 hr
				Endotoxin-control					
$\bar{P}pa$ mmHg	11.8 (2.2)	16.5 (9.2)	30.1* (10.6)	36.0* (7.3)	29.0* (4.8)	20.9 (6.6)	18.9 (3.0)	13.5 (1.2)	14.0 (3.6)
$\bar{P}paw$ mmHg	2.8 (0.5)	3.5 (1.9)	6.9* (3.9)	8.2* (4.3)	6.5* (3.3)	4.2 (3.4)	3.8 (1.5)	2.5 (0.6)	2.7 (0.6)
$\bar{P}sa$ mmHg	91 (13)	91 (12)	93 (13)	91 (13)	88 (14)	88 (10)	93 (12)	91 (14)	93 (14)
Q'p *l*/min	3.81 (0.4)	3.52 (0.5)	3.40 (0.4)	3.27 (0.8)	3.48 (0.7)	3.56 (0.3)	3.47 (0.4)	2.96* (0.4)	3.12 (0.4)
SVR mmHg *l*·min	24.0 (1.8)	26.0 (1.9)	27.5 (4.9)	29.3 (9.5)	24.5 (5.3)	24.9 (3.1)	26.7 (3.1)	31.1 (4.5)	30.1 (5.6)
Pao_2 mmHg	86 (9)	84 (10)	72 (10)	68* (8)	67* (13)	76 (15)	83 (12)	87 (11)	83 (11)
$Paco_2$ mmHg	34 (1.9)	35 (1.2)	37 (4.1)	36 (3.6)	36 (3.7)	36 (3.6)	34 (4.8)	34 (2.7)	36 (4.4)
pH units	7.52 (0.04)	7.52 (0.06)	7.51 (0.06)	7.50 (0.06)	7.51 (0.07)	7.51 (0.04)	7.55 (0.07)	7.55 (0.04)	7.55 (.03)
				FPL-57231 + Endotoxin					
$\bar{P}pa$ mmHg	13.3 (3.5)	14.1 (2.3)	†13.2 (3.3)	†15.2 (4.7)	†15.0 (3.6)	20.0 (7.2)	22.2* (5.9)	†21.4* (3.3)	†20.7* (4.1)
$\bar{P}paw$ mmHg	2.7 (0.7)	3.0 (0.8)	†2.3 (0.8)	†2.8 (1.3)	†3.0 (1.3)	3.0 (0.7)	3.8 (1.3)	4.2 (2.5)	4.4 (2.0)
$\bar{P}sa$ mmHg	93 (10)	91 (12)	87 (12)	89 (9)	87 (10)	97 (10)	101 (11)	101 (7)	99 (6)
Q'p *l*/min	3.54 (0.5)	4.34 (0.9)	4.19 (1.0)	†4.96* (1.4)	†4.93* (1.4)	3.29 (0.7)	2.82 (0.4)	2.66* (1.0)	3.05 (0.9)
SVR mmHg *l*·min	2.65 (2.7)	21.7 (4.8)	22 (6.1)	†19.6 (7.6)	19.4 (7.7)	30.2 (5.9)	†35.9 (2.1)	†40.5* (9.4)	33.5 (6.6)
Pao_2 mmHg	91 (16)	89 (9)	†87 (11)	†81 (11)	†80 (11)	†88 (13)	80 (14)	81 (21)	81 (22)
$Paco_2$ mmHg	36 (6.0)	35 (5.0)	35 (4.90	37 (4.8)	36 (5.2)	34 (4.9)	36 (7.5)	35 (7.7)	38 (8.4)
pH units	7.49 (0.08)	7.50 (0.07)	7.50 (0.08)	7.48 (0.06)	7.49 (0.06)	7.52 (0.06)	7.50 (0.08)	7.54 (0.01)	7.52 (0.01)

Data are shown as mean with SD in parenthesis. $\bar{P}pa$: mean pulmonary arterial pressure; $\bar{P}paw$: pulmonary arterial wedge pressure; $\bar{P}sa$:mean systemic arterial pressure; Q'p: cardiac output; SVR: systemic vascular resistance. *: $p<0.05$ Significantly different from baseline. †: Significantly different from endotoxin control. FPL–57231 (1 mg·kg·min) was administered during phase I (0–1 hour) only.

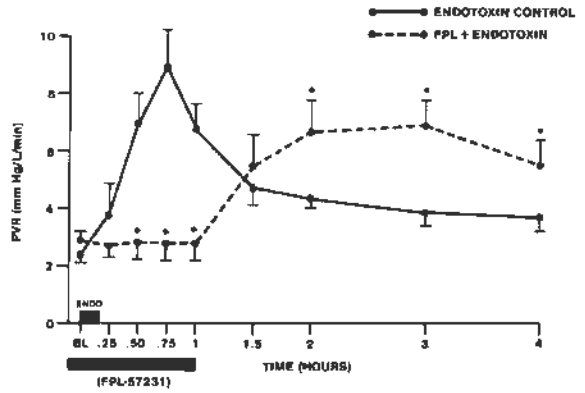

Fig. 1. Effect of one hour infusion during phase I of leukotriene inhibitor FPL-57231 (1 mg/kg/min) on endotoxin-induced (ENDO) changes in pulmonary vascular resistance (PVR). Data is shown as mean ± SE.
★ Significantly different from endotoxin control $p < 0.05$.

153–199%) of baseline, respectively. The changes in Ppa paralleled the changes in PVR, whereas Ppaw only increased during phase I (table I). Mean ± SE Pao_2 decreased from 86 ± 4 to 67 ± 6 mmHg during phase I and returned slowly towards baseline during phase II (table I). No significant changes were observed in Psa or SVR (table I). Arterial WBC count decreased from a mean of 8.6 ± 0.6 to 2.8 ± 0.7 thousand/mm^3 during phase I, and remained suppressed during phase II (fig. 2). Mean TxB_2 increased from 145 ± 28 to 3164 ± 1082 pg/ml, 6-Keto-$PgF_{1\alpha}$ increased from 129 ± 14 to 438 ± 114 pg/ml, and $PgF_{2\alpha}$ from 122 ± 7 to 242 ± 43 pg/ml (figs. 3–5). TxB_2 was significantly increased as early as 30 min after endotoxin, peaked at 1 h and then slowly returned towards basal level by 4 h. In contrast, 6-Keto-$PgF_{1\alpha}$ was not significantly different from baseline at 30 min, peaked between 1–2 h, and then returned to the basal levels by 3–4 h. $PgF_{2\alpha}$ was significantly different from baseline only at 1 h (figs 3–5).

B. Modification of endotoxin effects by FPL-57231

FPL-57231 infusion for one hour attenuated the phase I increases in Ppa, Ppaw and PVR; 45 min post-

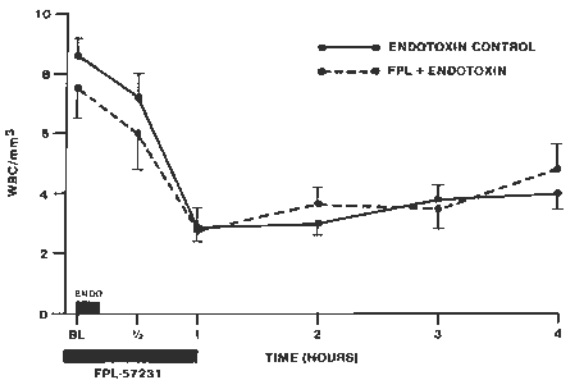

Fig. 2. Effect of FPL-57231 (1 mg/kg/min for one hour) infusion during phase I, on endotoxin-induced changes in white blood cell (WBC) count. Data is shown as mean ± SE.

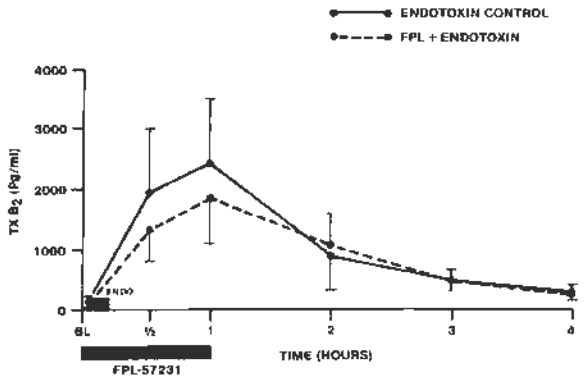

Fig. 3. Effect of FPL-57231 (1 mg/kg/min for one hour) infusion during phase I on endotoxin-induced increases in thromboxane B_2 (TxB_2). Data is shown as mean ± SE.

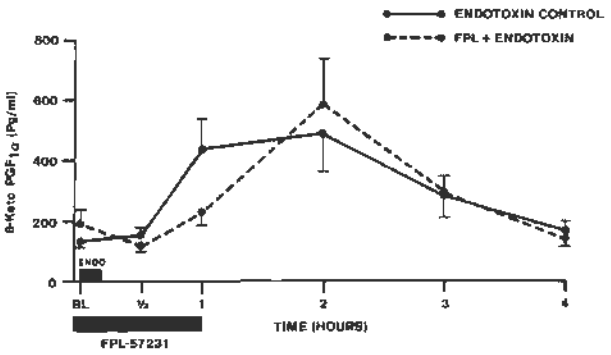

Fig. 4. Effect of FPL-57231 infusion (1 mg/kg/min for one hour) during phase I on endotoxin-induced increases in 6-Keto-$PgF_{1\alpha}$. Data is shown as mean ± SE.

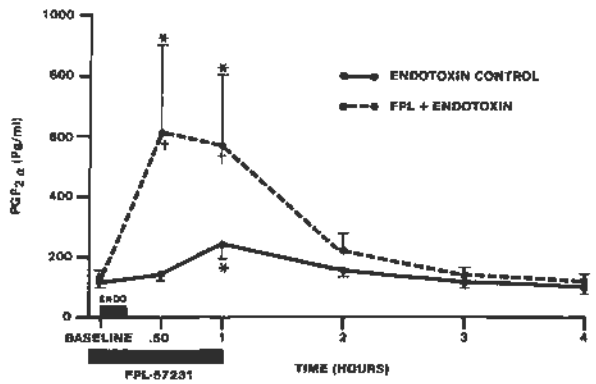

Fig. 5. Effect of FPL-57231 infusion (1 mg/kg/min for one hour) during phase I on endotoxin-induced increases in $PgF_{2\alpha}$. Data is shown as mean ± SE. * Significantly different from baseline $p < 0.05$; † Significantly different from endotoxin control $p < 0.05$.

endotoxin mean ± SE PVR was 94 ± 16% of baseline, which was not significantly different from baseline (fig. 1 and table I). However, discontinuation of FPL-57231 was followed by an exaggerated increase in Ppa and PVR during phase II (table I and fig. 1). Although Psa was not significantly changed following discontinuation of FPL-57231, an enhancement of SVR was observed during phase II (table I). FPL-

57231 prevented the endotoxin-induced decreases in PaO_2 (table I) but not in WBC count (fig. 2). FPL-57231 had no effect on the endotoxin-induced increases in TxB_2; one hour post-endotoxin mean ±SE TxB_2 increased to 2408±790 pg/ml, which was not different from that of endotoxin alone (fig. 3). FPL-57231 during phase I caused partial inhibition of endotoxin-induced increases in 6-Keto-$PgF_{1\alpha}$ (230±41 pg/ml), and enhanced $PgF_{2\alpha}$ generation (566±347 pg/ml) (figs 4 and 5). However, after discontinuation of FPL-57231, both the 6-Keto-$PgF_{1\alpha}$ and $PgF_{2\alpha}$ values were not different from the endotoxin control day.

Four hours post-endotoxin a 10 min infusion of FPL-57231 reversed the phase II increases in Ppa and PVR to basal levels (fig. 6 and table II); PVR decreased from 189±24% of baseline to 111±14% of baseline ($p < 0.05$). No significant changes in Ppaw and Psa were observed, whereas Q'p and SVR showed slight but significant changes (table II).

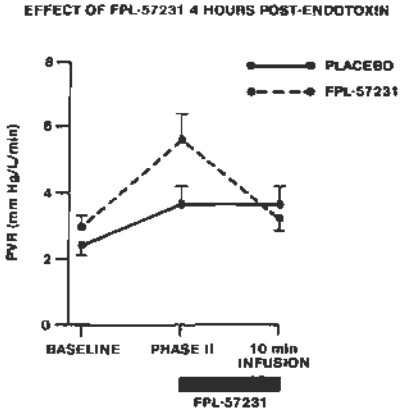

Fig. 6. Reversal of endotoxin-induced increases in PVR 4 h post-endotoxin by a 10 min infusion of FPL-57231 (1 mg/kg/min). FPL-57231 was administered at the end of phase II, *i.e.* 4 h post endotoxin. Data shown as mean±SE. Final PVR after 10 min FPL-57231 is significantly lower than value at start of infusion in this group.

Table II. - Reversal by a 10 min infusion of FPL–57321 (1 mg/kg/min) on endotoxin-induced changes in pulmonary and systemic haemodynamics, 4 hours post-endotoxin†

	Pre-Infusion	Post-Infusion
Ppa mmHg	20.7 (4.1)	15.3* (3.2)
Ppaw mmHg	4.4 (2.0)	3.5 (1.8)
Psa mmHg	99 (6)	96 (6)
Q'p *l*-min	3.05 (0.9)	3.57* (1.06)
PVR mmHg·*l*-min	5.57 (2.06)	3.35* (1.58)
SVR mmHg·*l*-min	33.5 (6.6)	28.2 (5.4)

† Data is shown as mean with SD in parentheses. Pre-infusion data is 4 hour post-endotoxin measurements as shown in Table I. Ppa: mean pulmonary arterial pressure; Ppaw: pulmonary arterial wedge pressure; Psa: mean systemic arterial pressure; Q'p: cardiac output; SVR: systemic vascular resistance. *: Significantly different from pre-infusion $p<0.05$

Discussion

Effects of exogenous endotoxin administration on pulmonary haemodynamics of sheep have been studied in detail previously [5, 6, 10, 28, 33]. Our findings are consistent with these studies and demonstrate that endotoxin causes biphasic response of PVR. Cyclooxygenase inhibitors, which inhibit the endotoxin-induced increases in TxB_2 and 6-Keto-$PgF_{1\alpha}$, attenuate the phase I increases in PVR [5, 10, 28, 33]. The fact that cyclooxygenase inhibitors only caused partial attenuation of PVR during phase I and had no effect during phase II, suggests that products of cyclooxygenase pathway are not the primary mediator of the pulmonary vascular response. Although an *in vivo* model has the limitation that flow, *i.e.* Q'p, cannot be controlled as in an *in vitro* perfused preparation, the results of the present investigation are consistent with the hypothesis that leukotrienes may play a pathophysiological role in the pulmonary vascular effects of endotoxin.

Four observations support the possibility that leukotrienes rather than thromboxane mediate the pulmonary vascular response, 1) the increase in PVR during phase II was temporarily not associated with an elevation of TxB_2 levels; 3 h post-endotoxin TxB_2 levels had nearly returned to the baseline while PVR was still elevated at 170% of baseline; 2) indomethacin, which inhibits the endotoxin-induced thromboxane generation does not prevent the phase II increases in PVR [5, 33]; 3) the increase in PVR during phase I is prevented by the leukotriene antagonist FPL-57231, which had no effect on thromboxane generation; 4) 4 h post-endotoxin during phase II a 10 min infusion of FPL-57231 reversed the phase II increase in PVR, when thromboxane had returned to the basal level.

FPL-57231 had no effect on endotoxin-induced increases in TxB_2, suggesting no cyclooxygenase inhibiting activity. However, there was blunting of 6-Keto-$PgF_{1\alpha}$ and enhanced generation of $PgF_{2\alpha}$ during phase I, suggesting that at the dose used FPL-57231 may have inhibited prostacyclin-synthetase. Selective inhibition of prostacyclin-synthetase could result in shunting of the enzyme pathway from prostacyclin-synthetase to endoperoxide F-reductase causing enhanced production of $PgF_{2\alpha}$. Similar observations were made with a 4-h infusion of FPL-57231 during both phases [5]. It is unlikely that FPL-57231

prevented the increase in PVR by blocking the effects of thromboxane or other cyclooxygenase products. Firstly, I.C.-50 of FPL-57231 for histamine, serotonin or $PgF_{2\alpha}$ is approximately 300–1200 fold higher than for SRS-A and LTD_4 [31]; and also *in vivo* FPL-57231 failed to modify the pulmonary vasoconstrictor effects of histamine and $PgF_{2\alpha}$ while attenuating that of LTD_4 [1, 3]. Secondly, FPL-57231 reversed the increases of PVR during phase II when thromboxane values were at basal levels. Whether FPL-57231 acted by inhibiting another unspecified mediator is not known at present.

In the present investigation, a continuous infusion of the leukotriene antagonist, FPL-57231 abolished the endotoxin-induced increases in PVR during phase I. When FPL-57231 was discontinued one hour post-endotoxin, PVR started to increase. This is not surprising because FPL-57231, when administered intravenously, has a very short half-life [32]. However, an unexpected finding during phase II was an exaggerated increase in Ppa and PVR after discontinuation of FPL-57231. The mechanism of this enhanced phase II following discontinuation of FPL-57231 is not clear. Since the 6-Keto-$PgF_{1\alpha}$ levels during phase II were not different from those of the endotoxin control, it is unlikely that enhancement of PVR during phase II was related to inhibition of prostacyclin (a vasodilator) production. It is possible that following discontinuation of the leukotriene antagonist infusion, the leukotriene receptors exhibited hyperresponsiveness to the agonist, thus resulting in exaggerated PVR response during phase II. It is also possible that FPL-57231 may have some other effects like lipoxygenase inhibiting activity during phase I and discontinuation of FPL-57231 may have resulted in enhanced production of leukotrienes during phase II.

We have recently observed that infusion of FPL-57231 for 4 h inhibited the increases in PVR during both phases [5]. Similarly, others have observed attenuation of endotoxin-induced changes in the pulmonary circulation of piglets with FPL-57231 [16] and sheep by LY171883 [19]. In contrast ZADOFF *et al.* were unable to attenuate pulmonary vascular effects of endotoxin by diethylcarbamazine [34], an agent which *in vitro* has been shown to inhibit the lipoxygenase pathway [22]. Since diethylcarbamazine inhibits both lipoxygenase and cyclooxygenase pathways, the failure of this agent to attenuate endotoxin-induced increases in cyclooxygenase products suggests that the dose of diethylcarbamazine in the ZADOFF *et al.* study [34] was perhaps inadequate. Although increases in 5-HETE in the lymph of sheep after endotoxin have been reported [27, 29], no measurable increases in LTC_4/LTD_4 were observed [13, 34]. This is perhaps related to relatively poor stability of leukotrienes in blood or lymph, as demonstrated by HAGMANN *et al.* [20], who observed rapid elimination of intravascular leukotrienes into bile following endotoxaemia in rats, resulting in increased levels of leukotrienes in bile extract. These authors also observed that leukotriene antagonists and/or 5-lipoxygenase inhibitors like FPL-55712, diethylcarbamazine, and BW755C, protected against the lethal action of endotoxin in mice, whereas cyclooxygenase inhibitors were ineffective [19].

Both indomethacin [5, 33] and FPL-57231 influence the increases in PVR during phase I, but have differential effects on thromboxane generation. In contrast, during phase II FPL-57231 effectively prevented the increase in PVR, while indomethacin was shown to be ineffective [5, 33]. Comparison of these data suggest that sustained increase of PVR during phase II may be predominantly mediated by leukotrienes, whereas the phase I increase is caused by a combination of cyclooxygenase and lipoxygenase products. It is also possible that phase II is caused by 'direct' (vascular leukotriene receptor stimulation) whereas phase I is related to both 'direct' and 'indirect' effects (secondary release of cyclooxygenase products). This concept is supported by our recent observation that the pulmonary and systemic haemodynamic effects of exogenous LTD_4 in sheep are mediated in part directly and in part indirectly via cyclooxygenase activation [1]. *In vitro* biochemical studies have also demonstrated that leukotrienes can release cyclooxygenase products [9, 11, 12, 26, 30].

Previous studies have shown that cyclooxygenase products do not mediate endotoxin-induced leucopenia, as cyclooxygenase inhibitors are ineffective [5, 33]. In the present investigation FPL-57231 which blocked LTC_4, LTD_4, and LTE_4 effects [2], failed to prevent leucopenia. Other mediators with chemotactic activity (for example LTB_4), which cause adherence of leucocytes to vascular endothelial cells, may be responsible [14]. Leukotriene B_4 is not antagonized by FPL-57231 and could therefore have caused leucopenia in our sheep. In contrast to our findings, COOK *et al.* [8] observed prevention of endotoxin-induced neutropenia in rats by another leukotriene antagonist, LY171883. This agent blocks LTD_4/LTE_4 but not LTB_4 receptors. Whether it acts like a 5-lipoxygenase inhibitor is not known.

Endotoxin-induced hypoxaemia was predominantly observed during phase I. Hypoxaemia, which is perhaps related to ventilation/perfusion imbalance, was effectively prevented by the leukotriene antagonist. Endotoxin causes marked alteration in airway mechanics as demonstrated by changes in pulmonary airflow resistance and dynamic lung compliance [5, 33]. Both FPL-57231 and indomethacin prevent the endotoxin-induced hypoxaemia and changes in airway mechanics, thus suggesting that hypoxaemia may be predominantly related to changes in ventilation [5, 33].

Leukotrienes have been suggested as the possible mediators of hypoxic pulmonary vasoconstriction in sheep [3], piglets [15], and isolated rat lungs [24, 25]. Increased levels of leukotrienes have been detected in lung lavage fluid of patients with acute lung injury [21] with altered lung microvascular permeability.

Although the results of the present investigation suggest a possible role of leukotrienes in endotoxin-induced pulmonary vasoconstriction, their role in altered microvascular permeability is not known.

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RÉSUMÉ: Nous avons investigué le rôle joué par les leucotriènes dans les modifications de la circulation pulmonaire induites par des endotoxines. Chez six moutons conscients nous avons mesuré les résistances vasculaires pulmonaires (PVR), ainsi que la Pao_2, la leucocytose (WBC), le thromboxane B_2 (TxB_2), 6-ceto-$PgF_{1\alpha}$ et $PgF_{2\alpha}$, avant et à des intervalles prédéterminés après une perfusion pendant 10 min d'endotoxine d'E coli (0,3 µg/kg); les mesures ont été réalisées sans et avec une prétraitement avec le FPL-57231, un antagoniste des récepteurs pour les leucotriènes. L'endotoxine induit une réponse biphasique (phase I ù 0–1 heure; phase II ù 5–4 heures) avec une augmentation de PVR (moyenne ± écart type de la moyenne) atteignant 415 ± 112% de la valeur basale pendant la phase I et une augmentation plus faible de 175% (étendue 153–199%) pendant la phase II. Pendant la phase I Pao_2 passe de 86 ± 4 à 67 ± 6 mmHg et la leucocytose chute de 8600 ± 600 à 2800 ± 700/mm^3, tandis que TxB_2 augmente de 145 ± 28 à 3164 ± 1082 pg/ml, 6-ceto-$PgF_{1\alpha}$ de 129 ± 14 à 438 ± 114 pg/ml et $PgF_{2\alpha}$ de 122 ± 7 à 242 ± 43 pg/ml. Une heure de perfusion de PPL-57231 (1 mg/Kg/min) administrée avant et pendant la phase I

atténue l'augmentation de PVR sans prévenir l'augmentation de TxB_2; pendant la phase I la production de 6-ceto-$PgF_{1\alpha}$ est atténuée tandis que celle de $PgF_{2\alpha}$ est favorisée. L'arrêt du FPL-57231 s'accompagne d'une exagération de la réponse en PVR pendant la phase II qui atteint en moyenne 209% de la valeur basale (étendue 186–235%). Quatre heures après l'administration de l'endotoxine, une perfusion de 10 min de FPL-57231 (1 mg/Kg/min) suffit pour ramener la PVR aux valeurs basales. FPL-57231 n'a aucun effet sur la diminution des leucocytes. Nous concluons que l'hypertension pulmonaire induite par une perfusion d'endotoxine peut être prévenue par et est réversible après l'administration d'un antagoniste des leucotriènes.