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Thromboxane Is Not Responsible for the High Pulmonary Vascular Resistance in Fetal Lambs

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ABSTRACT. The factors responsible for the high pulmonary vascular resistance in the fetus are not well known. Thromboxane (TX) A_2 is a potent pulmonary vasoconstrictor. To determine whether TXA_2 may play a role in fetal pulmonary vasoconstriction, we infused the specific TX synthetase inhibitor U63,557A into eight chronically instrumented fetal lambs (134–137 days gestational age, full term 145 days) and measured pulmonary blood flow, pulmonary and systemic arterial pressure, and heart rate. U63,557A (3 mg/kg as a bolus then 3 μg/kg/min for 120 min infused in the main pulmonary artery) did not change pulmonary blood flow, pulmonary mean arterial pressure, and pulmonary vascular resistance during the infusion and during 2 h following the end of the infusion. During the infusion, TXB_2 arterial plasma concentrations decreased from 106.1 ± 17.5 to 8.7 ± 2.9 pg/ml. In three of the fetal lambs, immediately after the 2-h infusion of U63,557A, we infused the leukotriene end-organ antagonist FPL 57231 into the main pulmonary artery (1 mg/kg/min for 60 min). TXA_2 synthesis inhibition did not prevent the pulmonary vasodilation induced by FPL 57231. Pulmonary blood flow increased from 64.8 ± 24.4 to 669.5 ± 65.6 ml/min/100 g lung tissue during the FPL 57231 infusion. We conclude that TXA_2 does not play a role in the maintenance of elevated fetal pulmonary vascular tone, either directly or as a mediator of leukotriene action. (*Pediatr Res* 19: 1254–1257, 1985)

Abbreviations

PG, prostaglandin
TX, thromboxane
PG 6 keto $F_1\alpha$, prostaglandin 6 keto $F_1\alpha$

The fetal circulation is characterized by a high pulmonary vascular resistance and a low pulmonary blood flow. In the near term fetal lamb, only 8 to 10% of the combined ventricular output is distributed to the pulmonary circulation (1). Several factors have been implicated in active pulmonary vasoconstriction in the fetus, however, no specific mechanism has yet been established to explain satisfactorily the normally high fetal pulmonary vascular resistance (2).

TXA_2, a metabolite of the cyclooxygenase pathway of arachidonic acid, is a potent pulmonary vasoconstrictor (3–6), which can be synthesized by adult (4, 6–10) and fetal (11, 12) lung. The enhanced release of TXA_2 is largely responsible for the pulmonary hypertension induced in newborn animals by endotoxin (13), group B streptococci (14), Intralipid (15), and exogenous arachidonic acid (16). In addition, TXA_2 may play a role in the pulmonary vasoconstriction induced in sheep by the peptidolipid leukotrienes, the 5-lipoxygenase metabolites of arachidonic acid (17).

Recently, the leukotriene end-organ antagonists FPL 55712 and FPL 57231 (Fisons, plc, Loughborough, England) have been shown in fetal lambs to induce a dramatic increase in pulmonary blood flow, suggesting a role for the leukotrienes in the maintenance of the high fetal pulmonary vascular resistance (18). However, the leukotrienes have both a direct vasoconstrictive effect on the pulmonary vasculature (19–21), as well as an indirect effect through the stimulation of TXA_2 production (22–25).

We undertook the present experiments to determine whether TXA_2 is involved in the maintenance of the normally high pulmonary vascular resistance of the fetus. We evaluated the effects of inhibition of TXA_2 synthesis by the specific TX synthetase inhibitor U63,557A on pulmonary blood flow in nonanesthetized chronically instrumented fetal lambs. We also attempted to determine whether inhibition of TXA_2 production would modify the previously observed (18) effects of the leukotriene receptor antagonist FPL 57231 on pulmonary blood flow.

MATERIALS AND METHODS

Surgical preparation. Eight fetal lambs were instrumented at 132–135 days gestation (full term is 145 days). Pregnant ewes were operated under low spinal anesthesia with 1% tetracaine hydrochloride and sedation with ketamine hydrochloride (100 mg intravenously when needed). The uterus was exposed through a midline abdominal incision and two uterine incisions were successively performed. Through the first one, a fetal hindlimb was exteriorized. Under local anesthesia (0.5% lidocaine HCl) polyvinyl catheters were inserted into the abdominal descending aorta through the pedal artery and into the inferior vena cava through the pedal vein. Succinylcholine chloride (10 mg) was then injected into the pedal vein to prevent fetal movements and the limb was returned into the uterine cavity. A catheter was introduced into the amniotic cavity and the uterine incision was sutured closed.

Through the second uterine incision, the fetal chest was opened in the third left intercostal space. A catheter was advanced into the ascending aorta through the internal mammary artery. Then the pericardium was opened and two catheters were directly placed, one into the pulmonary trunk and the other into the main pulmonary artery. The chest was sutured and the uterus closed. All the catheters were brought to a pouch sewn to the ewe's flank and the abdomen of the ewe was closed. Every day until the experiment, the ewe received 1 million U of penicillin G and 400 mg of kanamycin into the amniotic cavity and intravenously. The ewe and the fetal lambs were allowed to recover from surgery for 2 or 3 days.

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Measurement of pulmonary blood flow. Pulmonary blood flow was measured with 15 μm radionuclide labeled microspheres (26) labeled with eight different nuclides (selected from ^{153}Gd, ^{57}Co, ^{51}Cr, ^{113}Sn, ^{85}Sr, ^{95}Nb, ^{54}Mn, ^{65}Zn) injected into the inferior vena cava while withdrawing a reference sample from the pulmonary trunk at a rate of 4 ml/min for 1.5 min with a Harvard pump. Blood withdrawn was replaced with an equal volume of fetal blood from a twin or another fetal lamb. At the end of the experiment the ewe and fetus were killed with an overdose of sodium pentobarbital. The fetuses were weighed (3.43 ± 0.16 kg). The lungs were removed, weighed, and fixed in 10% formalin. The radioactivity for each isotope in both lungs and in the reference blood samples was measured by a well-type γ scintillation counter connected to a 1000-channel pulse-height analyzer (Ino-Tech, Norland Corp., Fort Atkinson, WI). Corrections for overlapping between isotopes were performed as previously described (27).

Measurement of hemodynamic variables. Systemic arterial pressure (abdominal aorta), pulmonary arterial pressure (pulmonary trunk), and intraamniotic pressure were measured with Statham P23 Db pressure transducers and recorded continuously on a Beckman Dynograph recorder. Intraamniotic pressure was used as a zero reference for systemic and pulmonary arterial pressures. Heart rate was measured with a cardiotachometer triggered from the arterial pressure wave. Since left atrial pressure was not measured directly, pulmonary vascular resistance was estimated as the ratio of mean pulmonary arterial pressure and pulmonary blood flow. To assess fetal state arterial blood gases, pH and oxygen saturation were measured from 0.4 ml abdominal aortic blood samples on a Corning model 158 blood gas analyzer and Radiometer OSM 2 hemoximeter.

Measurement of TXB_2 and PG 6 keto $F_1\alpha$. TXB_2, a stable metabolite of TXA_2, and PG 6 keto $F_1\alpha$, a stable metabolite of prostacyclin, were measured in 5 ml blood samples withdrawn from the ascending aorta into chilled, heparinized tubes which contained indomethacin (2 μg/ml blood). The blood was centrifuged at 2000 × *g* at 4° C for 20 min. One milliliter of plasma was mixed with 3000 dpm of ^{3}H-TXB_2 or ^{3}H-PG 6 keto F_1 (110–130 Ci/mmol New England Nuclear, Boston, MA) for calculation of recovery and acidified to pH 4.0 with citric acid. The plasma samples were extracted with a cyclohexane:ethyl acetate mixture, for TXB_2, or ethylacetate alone, for PG 6 keto $F_1\alpha$, and residues were stored at −20° C.

We determined TXB_2 or PG 6 keto $F_1\alpha$ by radioimmunoassay after their separation by silicic acid chromatography (28). For each concentration point of the standard curve for TXB_2 or PG 6 keto $F_1\alpha$ we carried a 1-ml sample of fetal lamb plasma, that had been incubated with activated charcoal to remove TXB_2 and PG 6 keto $F_1\alpha$ prior to extraction, through the purification process used for the 1-ml experimental plasma samples. Appropriate TXB_2 or PG 6 keto $F_1\alpha$ standards were added to each of these evaporated residues. This procedure yielded reproducible standard curves with linear titration from 1 to 100 pg TXB_2 and 25 to 500 pg PG 6 keto $F_1\alpha$. Antisera to TXB_2 and PG 6 keto $F_1\alpha$ were obtained from Seragen Inc., Boston, MA. Concentrations are expressed as free acid.

Experimental design. The experiment was performed while the ewe was standing in a cage with free access to food and water. During a control period, two determinations of baseline pulmonary blood flow, pressures, and blood gases were made separated by a 1-h interval. Then, the thromboxane synthetase inhibitor sodium 5-(3′-pyridinylmethyl) benzofuran-2-carboxylate (U63,557A) was administered into the main pulmonary artery first as a bolus [3 mg/kg estimated (using postmortem weights for fetuses of equivalent gestation) fetal body weight in 1.5 ml of saline] followed by a continuous infusion (3 μg/min/kg estimated body weight in 12 ml of saline for 120 min) (29, 30). Actual doses, based on weights obtained at the end of the study, were 3.3 mg/kg. In seven fetal lambs, the effects of U63,557A on pulmonary blood flow and hemodynamic variables were evalu-

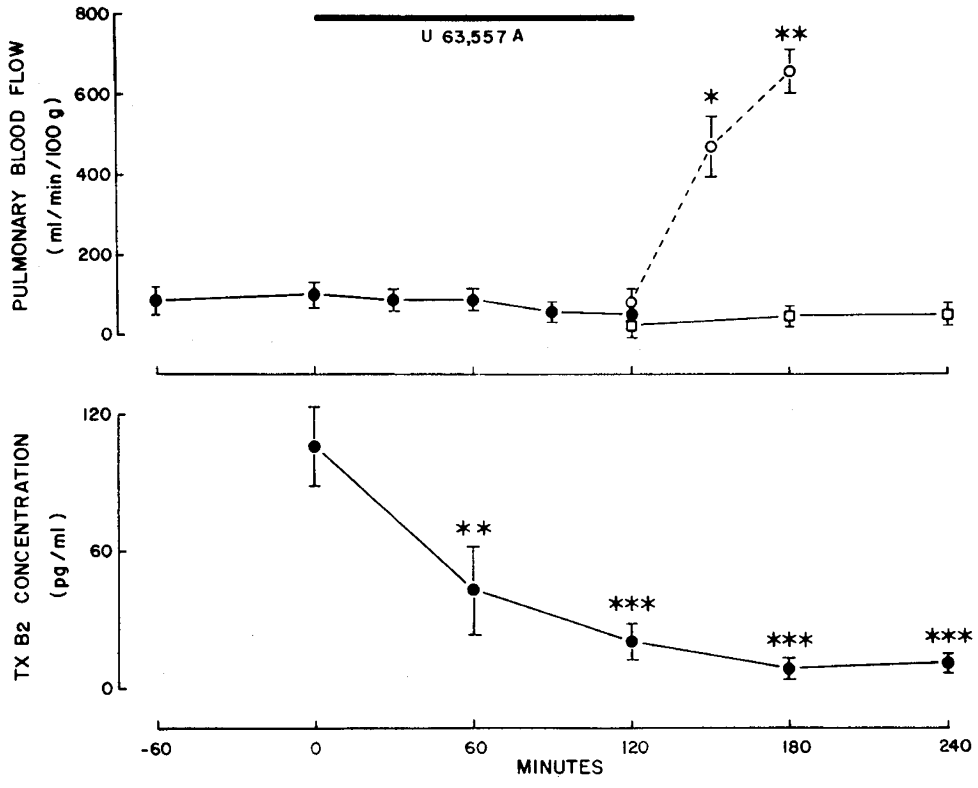

Fig. 1. Effect of U63,557A on pulmonary blood flow (seven fetal lambs) and TXB_2 (four fetal lambs) concentrations during control period (times -60 and 0 min) and during infusion of U63,557A (time 0 to 120 min). Values of pulmonary blood flow in five fetal lambs studied after cessation of U63,557A infusion (time 120 to 140 min) are represented by □——□. Values of pulmonary blood flow in three fetal lambs who received FPL 57231 after U63,557A are represented by O------O. * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$ compared to control.

ated at 30, 60, 90, and 120 min after the initial bolus dose. In five of the seven fetal lambs, a recovery period followed the end of the infusion, with determination of pulmonary blood flow and hemodynamic variables at 180 and 240 min. TXB_2 and PG 6 keto F_1 concentrations were measured before and 60, 120, 180, and 240 min after the beginning of the infusion in four and three fetal lambs, respectively.

In the two remaining fetal lambs and in one which received U63,557A but without sequential measurements during the infusion, we evaluated the effects of FPL 57231. FPL 57231 was infused (1 mg/min/kg estimated body weight in 6 ml sterile water for 60 min) starting immediately after the end of the U63,557A infusion (18). Pulmonary blood flow was measured before and 30 and 60 min after beginning FPL 57231.

Statistical analysis. Data were analyzed using a two-way analysis of variance for repeated measurements. Comparisons between means were made by Dunnett's and Newman-Keuls tests. Values are expressed as mean ± SEM.

RESULTS

Effects of U63,557A. The TX synthetase inhibitor U63,557A did not alter significantly the blood gases and pH or the pulmonary hemodynamics. Pulmonary blood flow (Fig. 1 and Table 1), pulmonary vascular resistance, and pulmonary arterial mean pressure (Table 1) were not significantly changed during the infusion of U63,557A and during the 2-h period following the end of the infusion. Systemic arterial mean pressure and heart rate (Table 1) were also unchanged during and after the infusion of U63,557A. There were no significant alterations in arterial blood gases and pH (Table 2).

U63,557A decreased plasma concentrations of TXB_2, from 106.1 ± 17.5 pg/ml before the infusion to 13.1 ± 7.3 pg/ml at 120 min and 8.7 ± 2.9 pg/ml at 180 min after the start of the infusion ($p < 0.001$) (Fig. 1). Plasma concentrations of PG 6 keto $F_1\alpha$ were not modified by administration of U63,557A (305.9 ± 45.3 pg/ml before the infusion and 294.8 ± 19.0, 243.0 ± 25.4, 260.5 ± 78.5, and 280.3 ±m 8.0 pg/ml, respectively, at 60, 120, 180, and 240 min after the start of the infusion).

Effects of FPL 57231 on fetal lambs pretreated with U63,557A. FPL 57231 increased pulmonary blood flow by 1033% from 64.8 ± 24.4 to 669.5 ± 65.6 ml/min/100 g ($p < 0.01$) in the three fetal lambs where TXA_2 production had been inhibited by U63,557A (Fig. 1 and Table 3). The increase in pulmonary blood flow was associated with a decrease in pulmonary vascular resistance from 1.25 ± 0.66 to 0.07 ± 0.01 mm Hg/ml/min/100 g.

DISCUSSION

The present results show that inhibition of TX synthetase does not affect the high pulmonary vascular resistance in near term fetal lambs. Moreover, the inhibition of TX synthetase does not prevent the dramatic increase in pulmonary blood flow induced by the leukotriene receptor antagonist FPL 57231 (18) and also does not have any major apparent systemic effect since heart rate, systemic blood pressure, and blood gases and pH were unaffected.

The inhibition of TX synthetase was achieved with U63,557A. This drug is a potent and specific inhibitor of TX synthetase (29, 30). We observed a 92% decrease in plasma arterial concentrations of TXB_2 following the administration of U63,557A. At the same time there were no significant changes in the concentration of PG 6 keto $F_1\alpha$, a metabolite of prostacyclin. Since the gradient between pulmonary and systemic arterial pressures was unchanged there appeared to be no effect of U63,557A on the ductus arteriosus (unlike cyclooxygenase inhibitors) (31).

Studies in adult animals suggest that TXA_2 may mediate reactive pulmonary vasoconstriction. The inhibition of TX synthetase by 1-pentylimidazole relaxes isolated rabbit intrapulmonary arteries (5). Moreover, exogenous arachidonic acid produces a vasoconstriction in adult guinea pig isolated lung and isolated rabbit pulmonary arteries which is mainly due to the release of TXA_2 (3, 5). However, our studies in normal fetal lambs indicate that TXA_2 does not contribute to the normally elevated pulmonary vascular resistance. This might be explained either by a lower responsiveness of fetal pulmonary arteries to TX, by differences in experimental design, or by a lower rate of pulmonary TXA_2 biosynthesis. A pulmonary vasoconstrictor effect of TXA_2 in fetuses was suggested by Tod and Cassin (16) who

Table 1. *Hemodynamic variables during control period and during infusion of U63,557A**

		Pulmonary arterial				
	Min	Mean pressure (mm Hg)	Blood flow (ml/min/100 g)	Vascular resistance (mm Hg/ml/min/100 g)	Systemic arterial mean pressure (mm Hg)	Heart rate (bpm)
Control period	−60	49.3 ± 1.6	86.5 ± 22.9	0.76 ± 0.13	48.6 ± 1.4	168 ± 5
	0	49.6 ± 1.8	101.0 ± 23.5	0.63 ± 0.11	49.9 ± 2.5	175 ± 5
U63,557A infusion	30	50.0 ± 1.7	85.4 ± 20.1	0.97 ± 0.32	50.0 ± 1.8	165 ± 6
	60	49.4 ± 1.4	88.0 ± 18.8	0.95 ± 0.35	52.0 ± 1.8	165 ± 9
	90	51.9 ± 1.2	60.2 ± 14.3	1.34 ± 0.39	51.1 ± 1.7	169 ± 8
	120	51.4 ± 1.9	51.8 ± 13.4	1.47 ± 0.34	50.6 ± 2.6	179 ± 8

* Data represent values during control period beginning 60 min before injection of U63,557A, and during infusion of the drug. There were no significant changes in any variables. Mean ± SEM; $n = 7$.

Table 2. *Arterial blood gases and pH measured in the abdominal aorta during the control period and during infusion of U63,557A**

	Minutes	pH	P_{CO_2} (torr)	P_{O_2} (torr)	O_2 Saturation
Control period	−60	7.35 ± 0.01	49.0 ± 1.5	19.0 ± 1.3	45 ± 2
	0	7.35 ± 0.01	49.0 ± 1.4	18.4 ± 1.5	43 ± 5
U63,557A infusion	30	7.35 ± 0.01	49.9 ± 1.2	19.0 ± 1.3	43 ± 6
	60	7.34 ± 0.01	50.0 ± 1.4	18.4 ± 1.4	41 ± 6
	90	7.33 ± 0.02	51.7 ± 1.6	17.9 ± 1.4	40 ± 6
	120	7.32 ± 0.02	50.3 ± 1.4	17.4 ± 1.7	39 ± 6

* Data represent values during control period beginning 60 min before injection of U63,557A and during infusion of the drug. There were no significant changes in any variables. Mean ± SEM; $n = 7$.

Table 3. *Effect of FPL 57231 infused over 60 min on hemodynamic variables in three fetal lambs pretreated with U63,557A**

	Min	Pulmonary arterial Mean pressure (mm Hg)	Pulmonary arterial Blood flow (ml/min/100 g)	Pulmonary arterial Vascular resistance (mm Hg/ml/min/100 g)	Systemic arterial mean pressure (mm Hg)	Heart rate (bpm)
FPL 57231 infusion	0	54.0 ± 1.5	64.8 ± 24.4	1.25 ± 0.66	54.3 ± 1.5	165 ± 8
	30	48.0 ± 2.5	481.5 ± 80.4†	0.10 ± 0.01	48.7 ± 0.7	205 ± 6
	60	45.3 ± 1.7	669.5 ± 65.6‡	0.07 ± 0.01	46.3 ± 0.7	220 ± 13

* Data represent values before (time 0) and at 30 and 60 min of infusion of FPL 57231. Mean ± SEM; $n = 3$. † $p < 0.02$, ‡ $p < 0.01$ compared to control (time 0).

observed that administration of exogenous arachidonic acid in ventilated fetal lambs produced a pulmonary vasoconstriction that was reversed by the TX synthetase inhibitor OKY-1581. Although TXA_2, synthesized after exogenous arachidonic acid administration, induces pulmonary vasoconstriction, this does not prove that TXA_2 plays a physiological role in maintaining the high fetal pulmonary vascular resistance. A lower rate of TXA_2 pulmonary synthesis has been previously shown in the fetal period compared to adult period using bovine and rabbit lung homogenates (11) and rabbit lung microsomes (12). The production of TXA_2 by fetal bovine lung homogenates represented less than 2% of the adult value (11). The rate of biosynthesis seemed to increase toward the end of gestation (11, 32).

Recent results from our laboratory have shown that FPL 57231, a leukotriene end-organ antagonist (33), induces a dramatic increase in pulmonary blood flow in chronically instrumented near term fetal lambs (18). The peptidolipid leukotrienes, mainly LTC_4 and LTD_4, are potent pulmonary vasoconstrictors in newborn lambs (34) and adult humans (20), monkeys (21), and guinea pigs (19). In certain circumstances their action may be dependent on the formation of TXA_2 in newborn lambs (34) and adult sheep (17). Our results demonstrate that TXA_2 does not play a significant role as a mediator of the leukotriene effect on the pulmonary vasculature of fetal lambs.

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