SURGERY FOR CONGENITAL HEART DISEASE

ENDOTHELIN RECEPTOR BLOCKADE PREVENTS THE RISE IN PULMONARY VASCULAR RESISTANCE AFTER CARDIOPULMONARY BYPASS IN LAMBS WITH INCREASED PULMONARY BLOOD FLOW

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Background: Children with increased pulmonary blood flow may experience morbidity as the result of increased pulmonary vascular resistance after operations in which cardiopulmonary bypass is used. Plasma levels of endothelin-1, a potent vasoactive substance implicated in pulmonary hypertension, are increased after cardiopulmonary bypass. **Objectives:** In a lamb model of increased pulmonary blood flow after in utero placement of an aortopulmonary shunt, we characterized the changes in pulmonary vascular resistance induced by hypothermic cardiopulmonary bypass and investigated the role of endothelin-1 and endothelin-A receptor activation in postbypass pulmonary hypertension. **Methods:** In eleven 1-month-old lambs, the shunt was closed, and vascular pressures and blood flows were monitored. An infusion of a selective endothelin-A receptor blocker (PD 156707; 1.0 mg/kg/h) or drug vehicle (saline solution) was then begun 30 minutes before cardiopulmonary bypass and continued for 4 hours after bypass. The hemodynamic variables were monitored, and plasma endothelin-1 concentrations were determined before, during, and for 6 hours after cardiopulmonary bypass. **Results:** After 90 minutes of hypothermic cardiopulmonary bypass, both pulmonary arterial pressure and pulmonary vascular resistance increased significantly in saline-treated lambs during the 6-hour study period ($P < .05$). In lambs pretreated with PD 156707, pulmonary arterial pressure and pulmonary vascular resistance decreased ($P < .05$). After bypass, plasma endothelin-1 concentrations increased in all lambs; there was a positive correlation between postbypass pulmonary vascular resistance and plasma endothelin-1 concentrations ($P < .05$). **Conclusions:** This study suggests that endothelin-A receptor-induced pulmonary vasoconstriction mediates, in part, the rise in pulmonary vascular resistance after cardiopulmonary bypass. Endothelin-A receptor antagonists may decrease morbidity in children at risk for postbypass pulmonary hypertension. This potential therapy warrants further investigation. (J Thorac Cardiovasc Surg 1999;117:314-23)

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Supported by grant 94-212 from the American Heart Association, California Affiliate, by Research Award 6-FY98-0615 from the March of Dimes, and by a grant from Parke-Davis Pharmaceutical Research.

Read at the Twenty-fourth Annual Meeting of The Western Thoracic Surgical Association, Whistler, British Columbia, June 24-27, 1998.

Received for publication July 7, 1998; revisions requested Aug 27, 1998; revisions received Sept 28, 1998; accepted for publication Oct 1, 1998.

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0022-5223/99 \$8.00 + 0 12/6/94979

The development of pulmonary hypertension is a common accompaniment of congenital heart disease with increased pulmonary blood flow.¹ Although early surgical repair of these congenital heart defects has decreased the incidence of irreversible pulmonary vascular disease, those children with reversible vascular changes experience morbidity and death in the postoperative period as the result of elevations in pulmonary vascular resistance and increased pulmonary vascular reactivity immediately after cardiopulmonary bypass (CPB).² Although increases in pulmonary vascular resistance and pulmonary vascular reactivity after CPB are well described, their mechanisms remain incompletely understood.

Recent evidence suggests that pulmonary vascular tone is regulated by a complex interaction of vasoactive substances that are locally produced by the vascular endothelium.³ During CPB, pulmonary vascular endothelial injury secondary to a variety of factors, including the disruption of normal pulmonary blood flow, complement activation, and neutrophil activation, may contribute to pulmonary hypertension after CPB.^{4,5} Endothelin-1 (ET-1) is a 21 amino acid polypeptide produced by vascular endothelial cells, the potent vasoactive properties of which have been implicated in the pathophysiology of pulmonary hypertensive disorders.⁶ The hemodynamic effects of ET-1 are mediated by at least 2 distinctive receptor populations, ET_A and ET_B.⁷ The ET_A receptors and a subpopulation of ET_B receptors mediate vasoconstriction and are located on vascular smooth muscle cells. A second subpopulation of ET_B receptors mediates vasodilatation and is located on vascular endothelial cells.⁷ In patients with congenital heart disease and pulmonary hypertension, plasma concentrations of ET-1 are increased immediately after CPB.⁸ Therefore alterations in ET-1 induced during CPB may be responsible, in part, for the increased pulmonary vascular resistance and increased vascular reactivity noted in children immediately after cardiac surgery.

We have established a model of pulmonary hypertension with increased pulmonary blood flow in the lamb, after in utero placement of an aorta-to-pulmonary vascular graft. At 1 month of age, these lambs (shunted lambs) have a pulmonary-to-systemic blood flow ratio of approximately 2:1, a mean pulmonary arterial pressure that is 75% of mean systemic arterial pressure, and pulmonary vascular remodeling characteristic of children with pulmonary hypertension and increased pulmonary blood flow.¹¹ The purpose of the present study was to use our animal model to characterize the changes in pulmonary vascular resistance induced by

hypothermic CPB and to specifically investigate the role of ET_A receptor activation in pulmonary hypertension after CPB. To characterize the changes in pulmonary vascular resistance induced by CPB, we monitored the hemodynamic response of the pulmonary circulation, in 5 one-month-old shunted lambs, for 6 hours after 90 minutes of hypothermic CPB. To assess the role of ET-1 and ET_A receptor activation in pulmonary hypertension after CPB, we determined the effects of ET_A receptor blockade on the hemodynamic response of the pulmonary circulation to CPB in an additional 6 shunted lambs and measured the plasma levels of ET-1 before and after CPB in all 11 lambs.

Methods

Surgical preparations and care

Ewes. Eleven mixed-breed Western pregnant ewes (139.7 ± 2.8 days of gestation; term, 145 days) were operated on under sterile conditions as previously described.⁹ Through a left lateral fetal thoracotomy, an 8.0 mm polytetrafluoroethylene* vascular graft (approximately 2 mm in length) was anastomosed between the ascending aorta and main pulmonary artery of the fetus with 7-0 proline (Ethicon Inc, Somerville, NJ), with a continuous suture technique as previously described.⁹ After recovery from anesthesia, the ewe was returned to the cage with free access to food and water. Antibiotics (2 million units of penicillin G potassium and 100 mg of gentamicin sulfate) were administered to the ewe during the operation and daily thereafter until 2 days after spontaneous delivery of the lamb.

Lambs. After spontaneous delivery, the lambs were treated with antibiotics (1 million units of penicillin G potassium and 25 mg of gentamicin sulfate, intramuscularly) for 2 days. The lambs were weighed daily, and the respiratory rate and heart rate were obtained. Furosemide (1 mg/kg, intramuscularly) was administered daily. Elemental iron (50 mg, intramuscularly) was given weekly.

At 1 month of age, 11 shunted lambs were anesthetized with ketamine hydrochloride (approximately 1 mg/kg/min) and mechanically ventilated as previously described.^{10,11} Through a midsternotomy incision, polyurethane catheters were inserted into the left and right atria and the main pulmonary artery distal to the vascular graft. Ultrasonic flow probes (Transonics Systems Inc, Ithaca, NY) were placed around the left and right pulmonary arteries to measure pulmonary blood flow. After a 30-minute recovery, blood was obtained from the left and right atria, distal pulmonary artery, right ventricle, and descending aorta for hemoglobin and oxygen saturation determinations. A vascular clamp was then placed on the graft to completely occlude it, and oxygen saturation determinations were obtained from the right ventricle and distal pulmonary artery to document shunt closure. The sternum was then tem-

*W.L. Gore and Associates, Inc, Newark, Del.

porarily approximated. An intravenous injection of 250,000 units of penicillin G potassium and 25 mg of gentamicin sulfate suspension was administered.

CPB. The bypass circuit is similar to the standard neonatal circuit.¹² It consists of a membrane oxygenator (Minimax; Medtronic, Inc, Grand Rapids, Mich), an infant venous reservoir (Medtronic, Inc), an arterial filter (40 μ m; Bentley, Irvine, Calif), and a cardiomy reservoir and suction. An ultrasonic flow probe (Transonics Systems Inc) is incorporated into the circuit to monitor pump flows continuously. The circuit is primed with fresh heparinized sheep whole blood (400 mL), crystalloid (Normosol; 600 mL), heparin (2500 units), sodium bicarbonate (10 mEq), methylprednisolone sodium succinate (Solu-Medrol; 30 mg/kg), and cefazolin (Kefzol; 25 mg/kg). The bypass method is similar to standard neonatal methods.¹² Heparin (300 U/kg) was administered to the lamb in the right atrium. Bicaval venous cannulation was performed with a 16F venous cannula (DLB Inc, Grand Rapids, Mich) for the superior vena cava and a 20F venous cannula for the inferior vena cava. The ascending aorta was cannulated with a 14F cannula (Electro-catheter Corp, Rahway, NJ). CPB was begun, and surface and core cooling was initiated. The sternum was again temporarily approximated. Normothermic flows ranged from 150 to 200 mL/kg/min. The lambs were cooled to 25°C, and flow was reduced to 100 mL/kg/min. After 60 minutes at 25°C, rewarming was started. Throughout the CPB period, an alpha stat blood gas strategy was maintained, whereby the temperature-uncorrected P_{aCO_2} is maintained near 40 mm Hg (measured at 37°C) and the temperature-uncorrected pH is maintained near 7.40, irrespective of body temperature.¹³ Mannitol (0.5 gm/kg) and furosemide (0.5 mg/kg) were added to the prime at the onset of rewarming. After the core temperature reached 32°C, calcium gluconate (1 g) was added to the prime. After the lambs were rewarmed, ventilation was resumed and CPB was weaned off. Heparin was completely reversed with protamine (3 mg/kg) given into the left atrium.

Measurements. Pulmonary and systemic arterial and right and left atrial pressures were measured with pressure transducers (P23Db; Statham Instruments, Hato Rey, Puerto Rico). Mean pressures were obtained by electrical integration. Heart rate was measured by a cardiometer triggered from the phasic systemic arterial pressure pulse wave. Left and right pulmonary blood flow and bypass flows were measured on an ultrasonic flow meter (Transonic Systems Inc). All hemodynamic variables were recorded continuously on a multichannel electrostatic recorder (Gould Inc, Cleveland, Ohio). Systemic arterial blood gases and pH were measured on a pH/blood gas analyzer (Corning 158; Corning Medical and Scientific, Medfield, Mass). Hemoglobin concentration and oxygen saturation were measured by a hemoximeter (model OSM 2; Radiometer, Copenhagen, Denmark). The ratio of pulmonary to systemic blood flow was calculated with the Fick equation. After shunt closure, systemic blood flow was defined as total pulmonary blood flow. Pulmonary and systemic vascular resistances were calculated with standard formulas.

ET-1 determinations. Four milliliters of systemic arterial

blood was collected and placed in iced polypropylene tubes containing 330 μ L aprotinin and 100 μ L EDTA. The tubes were immediately centrifuged at 4000g for 20 minutes. Collected plasma was treated with equal volumes of 0.1% trifluoroacetic acid and stored at -70°C. The acidified supernatant was centrifuged at 1000g for 20 minutes and loaded on a 3 $\times$ 18 C18 Sep-Pak column (Peninsula Laboratories, Belmont, Calif) equilibrated with 0.1% trifluoroacetic acid. The adsorbed material was eluted with 3 mL of 0.1% trifluoroacetic acid/60% acetonitrile. The eluant was dried in a Savant speed vacuum and stored at -70°C or assayed immediately for immunoreactive endothelin. ET-1 standard, ET-1 labeled with iodine 125, anti-ET antibody, and secondary antibody were purchased from Peninsula Laboratories, Belmont, Calif. Cross-reactivity for measured human and bovine ET-1 antiserum is 100% for human ET-1, 7% for human ET-2 and ET-3, and 0% for bovine ET-2 and ET-3. Interassay and intra-assay variabilities were 10% and 4%, respectively. Plasma levels were not corrected for hemodilution.

PD 156707 determinations. Two milliliters of systemic arterial blood were collected and immediately centrifuged at 4000g for 20 minutes. Collected plasma was stored at -70°C. Plasma concentrations were determined by a liquid chromatographic assay, as previously described.¹⁴

Experimental protocol. Sixty minutes after shunt and chest closure, baseline measurements of the hemodynamic variables (pulmonary and systemic arterial pressure, heart rate, pulmonary blood flow, left and right atrial pressures), and systemic arterial blood gases and pH were measured. Then an infusion of PD 156707, a selective ET_A receptor blocker (1 mg/kg/h; synthesized by the Medicinal Chemistry Department, Parke-Davis Pharmaceutical Research, Ann Arbor, Mich), or drug vehicle (saline solution; randomly selected) was begun and continued during CPB and for 4 hours after CPB.¹⁵ The dose of PD 156707 was chosen after several preliminary studies showed that a 30-minute infusion completely blocked the vasoconstricting effects of exogenous ET-1 and resulted in steady-state plasma concentrations that blocked ET_A receptors in vivo.¹⁶ Thirty minutes after the initiation of the infusion, all measurements were repeated (before bypass) and CPB was begun. The hemodynamic variables were monitored continuously during and for 6 hours after CPB. Systemic arterial blood gases were determined intermittently, and ventilation was adjusted to achieve a P_{aCO_2} between 35 and 45 mm Hg and a P_{aO_2} of more than 50 mm Hg. Sodium bicarbonate was administered intermittently to maintain a pH of more than 7.30. Normal saline solution and red blood cells were administered to maintain atrial pressures and hemoglobin concentrations at pre-CPB levels. Blood from the femoral artery was obtained for ET-1 levels before and 0.5, 1, 2, 4, and 6 hours after CPB. Blood was obtained for plasma PD 156707 concentrations before and 4 and 6 hours after CPB. Four hours after CPB, the infusion of PD 156707 was stopped, and the hemodynamic variables were monitored for an additional 2 hours. All lambs were killed with a lethal injection of sodium pentobarbital followed by bilateral thoracotomy as described in the NIH

Table I. General hemodynamics

	Saline-treated shunted lambs (n = 5)	PD 156707-treated shunted lambs (n = 6)
Age (days)	28.7 ± 2.5	32.8 ± 7.6
Weight (kg)	10.4 ± 1.3	11.8 ± 2.9
Hemoglobin (g/dL)	9.6 ± 1.6	9.1 ± 1.0
Mean pulmonary arterial pressure (mm Hg)	35.6 ± 6.1	29.2 ± 6.8
Mean systemic arterial pressure (mm Hg)	56.0 ± 12.1	59.3 ± 11.2
Pulmonary vascular resistance (mm Hg/mL/min/kg)	0.118 ± 0.06	0.087 ± 0.03
Pulmonary blood flow (mL/kg/min)	250.4 ± 81.7	241.3 ± 63.4
Heart rate (beats/min)	165.4 ± 26.3	161.2 ± 22.4
Left atrial pressure (mm Hg)	8.7 ± 3.8	9.3 ± 2.6
Right atrial pressure (mm Hg)	7.7 ± 1.5	5.8 ± 1.5
Pulmonary artery pressure/systemic artery pressure	0.66 ± 0.17	0.51 ± 0.15
Pulmonary blood flow/systemic blood flow	2.7 ± 1.0	3.2 ± 1.3
CPB time (min)	114.2 ± 12.8	131.7 ± 18.3

Values are mean ± SD; values in shunted lambs were obtained before closure of the vascular graft. There were no differences between the 2 groups.

Table II. The hemodynamic changes after CPB in saline-treated shunted lambs (n = 5)

	Before CPB	Time after CPB (min)							
		15	30	60	120	180	240	300	360
Pulmonary arterial pressure (mm Hg)	27.2±5.5	40.2±9.8*	36.2±7.9*	35.6±9.7*	35.4±1.8*	32.2±6.3*	33.8±6.1*	33.2±6.0*	30.0±5.8*
Pulmonary vascular resistance (mm Hg/mL/min/kg)	0.148±0.06	0.309±0.16*	0.263±0.13*	0.252±0.14*	0.280±0.13*	0.280±0.18*	0.288±0.19	0.296±0.20*	0.193±0.09
Pulmonary blood flow (mL/kg/min)	127.5±18.30	109.2±40.9	112.81±44.8	110.8±29.4	103.4±32.9	85.5±21.9*	92.1±28.7	89.8±28.3	99.6±26.4
Systemic arterial pressure (mm Hg)	67.6±23.4	53.4±3.9*	53.2±12.3*	57.8±9.6	60.0±27.3	45.6±20.7*	44.2±17.1*	39.4±13.9*	40.5±10.2*
Heart rate (beats/min)	157.8±26.6	145.0±16.0	141.8±15.8	139.6±18.9	149.6±27.5	149.6±26.9	147.4±38.4	143.4±30.7	154.0±19.2
Left atrial pressure (mm Hg)	9.0±0.0	10.8±1.9	10.2±2.1	9.8±2.1	9.8±1.0	10.8±1.6	11.2±2.6	10.4±3.9	11.0±4.2
Right atrial pressure (mm Hg)	9.8±3.0	10.6±1.1	10.2±1.6	9.4±1.1	8.8±1.9	9.9±1.7	10.2±1.9	9.2±2.8	9.5±3.1
pH (units)	7.38±0.08	7.34±0.09	7.34±0.08	7.36±0.08	7.27±0.07*	7.31±0.04*	7.31±0.04*	7.31±0.05*	7.35±0.04
Paco ₂ (mm Hg)	42.0±6.4	38.0±6.9	37.6±5.2	38.4±9.9	49.2±10.4	43.2±5.5	44.4±8.4	39.4±5.1	40.3±8.7
PaO ₂ (mm Hg)	58.0±5.8	68.4±11.2	83.8±18.7	74.6±27.6	82.2±57.5	70.6±26.1	68.6±19.2	62.8±16.9	59.5±14.1

Values are mean ± SD.

*P < .05 vs before CPB (ANOVA).

Guidelines for the Care and Use of Laboratory Animals. All protocols and procedures were approved by the Committee on Animal Research of the University of California, San Francisco.

Statistical analysis. The means ± standard deviation were calculated for the baseline hemodynamic variables, systemic arterial blood gases and pH, and plasma ET-1 and PD 156707 concentrations. The baseline hemodynamic variables were compared between groups by the unpaired *t*-test. The general hemodynamic variables, systemic arterial blood gases and pH, and ET-1 concentrations were compared over time within each study group by analysis of variance (ANOVA) for repeated measures with an autoregressive

symmetric covariance structure. Adjustments for multiple comparisons from baseline were made by the Dunnett procedure. The Tukey procedure was used for multiple comparisons across groups. The results from both groups were pooled, and a linear regression analysis of the pulmonary vascular resistance and mean pulmonary arterial pressure with plasma ET-1 concentrations was performed to identify possible relevant relations.

Results

The general hemodynamics of the 2 groups of lambs before the initiation of CPB are presented in Table I.

Fig 1. The infusion of PD 156707 resulted in steady-state plasma concentrations; these decreased over the 2 hours after the infusion was discontinued. Values are mean $\pm$ SD; $n = 5$.

All lambs had an increase in oxygen saturation between the right ventricle and distal pulmonary artery. There were no differences in the general hemodynamics between the 2 groups. In addition, systemic arterial blood gases and pH were similar in both groups, and all were within the normal range for the laboratory (data not shown).

In saline-treated shunted lambs, both pulmonary vascular resistance ($P = .023$) and mean pulmonary arterial pressure ($P = .002$) increased over the 6-hour study period after CPB. Pulmonary blood flow, mean systemic arterial pressure, and systemic arterial pH were intermittently decreased. Heart rate, left and right atrial pressure, $Paco_2$, and Pao_2 were unchanged (Table II).

The intravenous infusion of PD 156707 decreased mean systemic arterial pressure from 61.3 ± 14.1 mm Hg to 52.5 ± 12.8 mm Hg ($P < .033$). All other hemodynamic variables were unchanged. Over the 4.5-hour infusion period, plasma PD 156707 concentrations remained over 500 ng/mL (from 546.5 ± 125.0 ng/mL to 580.0 ± 211.0 ng/mL). Two hours after discontinuation of the infusion, plasma concentrations decreased to 97.2 ± 63.2 ng/mL (Fig 1). In PD 156707-treated shunted lambs, pulmonary vascular resistance significantly decreased after CPB ($P = .0001$). Mean pulmonary arterial pressure was unchanged after CPB (P

Fig 2. PD 156707 blocks the increase in mean pulmonary arterial pressure (top) and pulmonary vascular resistance (bottom) in shunted lambs immediately after CPB. Values are mean $\pm$ SD; $n = 5$ saline-treated shunted lambs; $N = 6$ PD 156707-treated shunted lambs. $*P < .05$ vs before CPB (time 0; ANOVA).

$= .493$). Pulmonary blood flow increased, and mean systemic arterial pressure decreased after 240 minutes. Heart rate, left and right atrial pressure, systemic arterial pH, $Paco_2$ and Pao_2 were unchanged (Table III). After CPB, both pulmonary vascular resistance and mean pulmonary arterial pressure were greater in saline-treated shunted lambs than in PD 156707-treated shunted lambs (Table IV) (Fig 2). After discontinuation of the PD 156707 infusion (4 hours after CPB), mean pulmonary arterial pressure and pulmonary vas-

Table III. The hemodynamic changes after CPB in PD 156707-treated shunted lambs ($n = 6$)

	Before CPB	Time after CPB (min)							
		15	30	60	120	180	240	300	360
Pulmonary arterial pressure (mm Hg)	22.0±3.5	19.8±4.3*	18.8±3.4*	17.7±3.3*	19.0±5.6	18.5±4.3*	18.7±5.0*	19.0±3.9*	19.8±3.9
Pulmonary vascular resistance (mm Hg/mL/min/kg)	0.186±0.05	0.074±0.03*	0.078±0.03*	0.085±0.03*	0.128±0.06*	0.108±0.03*	0.106±0.04*	0.107±0.05*	0.122±0.07*
Pulmonary blood flow (mL/kg/min)	84.8±5.3	150.9±20.9*	132.3±28.7*	121.2±29.6*	101.7±25.0*	105.5±20.7*	109.3±28.4*	115.9±26.5*	118.1±32.8
Systemic arterial pressure (mm Hg)	52.5±12.8	60.8±8.9*	57.0±9.0	55.8±8.7	51.1±4.7	49.8±10.2	45.1±9.4*	45.3±6.3*	43.1±8.1*
Heart rate (beats/min)	151.8±6.9	154.0±21.8	149.3±19.9	150.8±20.2	154.7±16.7	152.8±12.0	157.7±11.2	159.8±16.4	166.8±18.9*
Left atrial pressure (mm Hg)	6.4±2.0	8.5±2.2*	8.8±3.2*	7.7±2.1*	6.7±2.1	7.3±2.1	7.3±1.9	7.2±2.3	6.7±2.3
Right atrial pressure (mm Hg)	5.6±2.2	8.5±1.9*	8.6±2.2*	6.8±1.5	6.7±1.5	6.8±1.3	7.0±1.2*	6.6±1.6	6.5±1.6
pH (units)	7.33±0.03	7.32±0.07	7.34±0.08	7.36±0.05	7.38±0.04	7.37±0.05	7.39±0.04*	7.38±0.05	7.38±0.07
Paco ₂ (mm Hg)	45.5±5.5	44.3±15.0	45.3±11.1	43.0±8.1	41.5±5.0	43.5±6.9	41.7±5.5	43.5±5.0	44.0±8.1
Pao ₂ (mm Hg)	59.5±11.4	109.1±75.4*	87.1±39.1	73.8±13.0	61.0±6.6	52.5±5.3	52.8±2.3	50.5±4.5	49.8±1.5

Values are mean ± SD.

* $P < .05$ vs before CPB (ANOVA).

Table IV. ANOVA comparison of P values of the mean pulmonary arterial pressure of PD156707-treated vs saline-treated lambs

PD 156707-treated shunted lambs ($n=6$) after CPB (min)	Saline-treated shunted lambs ($n=5$) after CPB (min)								
	0	15	30	60	120	180	240	300	360
0	.981	.001	.006	.012	.014	.193	.059	.095	.403
15	.732	.000	.001	.001	.002	.036	.008	.015	.108
30	.521	.000	.000	.000	.001	.015	.003	.006	.05
60	.292	.000	.000	.000	.000	.005	.000	.002	.019
120	.575	.000	.000	.001	.001	.019	.004	.007	.061
180	.450	.000	.000	.000	.000	.011	.002	.004	.038
240	.485	.000	.000	.000	.000	.013	.003	.005	.044
300	.557	.000	.000	.001	.001	.017	.004	.007	.057
360	.732	.000	.001	.001	.002	.037	.008	.015	.108

cular resistance did not change. Throughout the study period, the mean airway pressure, minute ventilation, and FiO_2 required to maintain the predetermined ventilatory parameters were similar in saline-treated shunted lambs and in PD 156707-treated shunted lambs.

ET-1 concentrations. Before CPB, plasma ET-1 concentrations were similar in both groups of lambs. After CPB, ET-1 concentrations increased in both groups of lambs (Fig 3). One and 2 hours after CPB, ET-1 concentrations were greater in saline-treated shunted lambs than in PD 156707-treated shunted lambs ($P < .05$). There was a positive correlation between plasma ET-1 concentrations and pulmonary vascular resistance ($r =$

0.3; $P = .0024$) and mean pulmonary arterial pressure ($r = 0.4$; $P = .0001$).

Discussion

The present study provides support for a role for ET-1 and ET_A receptor activation in the pathogenesis of pulmonary hypertension after CPB. Pulmonary vascular resistance, mean pulmonary arterial pressure, and plasma ET-1 concentrations were increased after hypothermic CPB in 1-month-old lambs with pre-existing increased pulmonary blood flow. The intravenous infusion of PD 156707, a selective ET_A receptor antagonist, completely blocked the increase in pulmonary vascular

Fig 3. After CPB, plasma ET-1 concentrations are increased in all lambs. At 1 and 2 hours, plasma ET-1 is significantly higher in the saline-treated shunted lambs. Values are mean $\pm$ SD; $n = 5$ saline-treated shunted lambs; $n = 6$ PD 156707-treated shunted lambs. * $P < .05$ vs before CPB (time 0; ANOVA).

resistance and mean pulmonary arterial pressure after CPB. The increase in plasma ET-1 concentration after CPB correlated with the increase in pulmonary vascular resistance and mean pulmonary arterial pressure.

An association between ET-1 and pulmonary hypertensive disorders in humans has been well described. For example, several studies demonstrate increased ET-1 concentrations in children with congenital heart disease that is associated with increased pulmonary blood flow and pulmonary hypertension.¹⁷ In addition, patients with advanced pulmonary hypertension secondary to a variety of conditions display increased ET-1 gene expression within the pulmonary vascular endothelial cells.¹⁸ Recent studies have suggested a role for ET-1 in the pathophysiologic evidence for pulmonary hypertension after CPB. For example, in children with congenital heart disease and pulmonary hypertension, plasma concentrations of ET-1 are increased immediately after CPB.⁸ In addition, in normal piglets, inhibition of endothelin-converting enzyme-1 (the enzyme that converts proendothelin into its functional form) attenuates pulmonary hypertension after CPB.⁹

The hemodynamic effects of ET-1 are mediated by at least 2 distinctive receptor populations, ET_A and ET_B,

the densities of which are different depending on the vascular bed studied. The ET_A receptors are located on vascular smooth muscle cells and mediate vasoconstriction, whereas the ET_B receptors may be located on endothelial cells and mediate both vasodilation and vasoconstriction.⁷ In addition, a second subpopulation of ET_B receptors is located on smooth muscle cells and mediate vasoconstriction.²⁰ In lambs with pre-existing increased pulmonary blood flow, the infusion of PD 145065 (a combined ET_A and ET_B receptor antagonist) completely blocked the increase in pulmonary vascular resistance after hypothermic CPB.¹⁰ However, the ET-1 receptor subtype that mediates pulmonary vasoconstriction after CPB has been unclear. A recent study in piglets demonstrates a increase in ET_B receptor expression in lung parenchyma after hypothermic CPB, suggesting a possible role for ET_B receptor-mediated pulmonary vasoconstriction.²¹ In the present study, we have shown that selective ET_A receptor blockade completely blocks pulmonary vasoconstriction after CPB, suggesting that pulmonary hypertension after CPB is mediated, at least in part, through the ET_A receptor activation.

To selectively block ET_A receptor activity during and after CPB, we used PD 156707, a non-peptide ET_A receptor antagonist. PD 156707 is highly selective for the ET_A receptor and inhibits the binding of ¹²⁵I-ET-1 to cloned human ET_A receptor and ET_B receptor with K_i values of 0.17 and 133.8 nmol/L, respectively.¹⁵ In rabbits, PD 156707 infusion rates of 0.03 mg/kg/h completely blocked the vasoconstricting effects of exogenous ET-1, with corresponding plasma concentrations that were less than 0.05 μ g/mL (10^{-7} mol/L).^{14,16} We have also performed several preliminary studies in lambs that demonstrate that PD 156707 infusion rates of 1.0 mg/kg/h completely and selectively block the vasoconstricting effects of exogenous ET-1 (250 ng/kg) and produce stable plasma concentrations within 30 minutes of initiating the infusion (data not shown). Therefore in the present study we used an infusion rate of 1.0 mg/kg/h that was initiated 30 minutes before the initiation of CPB. As demonstrated in Fig 1, these infusions resulted in stable plasma concentration of more than 500 ng/mL throughout the infusion period.

After the discontinuation of the PD 156707 infusion, plasma concentrations quickly decreased to subtherapeutic values. We monitored the hemodynamic variables for 2 hours after discontinuation of the infusion and found that despite persistently increased plasma ET-1 concentrations, pulmonary vascular resistance and pulmonary arterial pressure did not increase. This most likely represents prolonged ET_A receptor block-

ade and/or other alterations in vascular reactivity induced by prolonged ET_A receptor blockade. For example, several studies demonstrate physiologic interactions between the ET-1, nitric oxide, and the prostaglandin pathways.²²

After CPB, plasma ET-1 concentrations increased in both groups of lambs. This has been previously noted in patients with pulmonary hypertension and congenital heart disease.⁸ The cause of increased ET-1 concentrations after CPB is unknown, but several factors associated with CPB (eg, surgical stress, hypothermia, alveolar hypoxia, and cardiogenic shock) are known to increase ET-1 concentrations.²³⁻²⁵ Because plasma concentrations of ET-1 increased more in saline-treated lambs than in PD 156707-treated lambs, there was a positive correlation between the increase in pulmonary vascular resistance after CPB and the plasma ET-1 concentration. However, this positive correlation has been inconsistent in other studies. For example, we have previously demonstrated that normal lambs and shunted lambs have similar increases in plasma ET-1 concentrations after CPB, but shunted lambs have a much greater increase in pulmonary vascular resistance.¹⁰ Although circulating ET-1 concentrations are a marker of ET-1 production, circulating ET-1 levels may be less important than the higher tissue concentrations achieved by local ET-1 release.²⁶ In addition, the physiologic effects of ET-1 are dependent on the pre-existing status of the pulmonary circulation.^{11,27} For example, increased pulmonary blood flow and pulmonary hypertension are associated with increased ET_A receptor and decreased ET_B receptor expression.^{11,27} Therefore significantly greater ET-1-induced pulmonary vasoconstriction may occur under these conditions even if plasma and local tissue concentrations are similar.

Previously, we have shown that the increase in pulmonary vascular resistance after CPB is dependent on the pre-existing status of the pulmonary circulation; pulmonary vascular resistance increased in shunted lambs after CPB but remained unchanged in age-matched control lambs.¹⁰ Therefore we used shunted lambs for the current study. However, it should be noted that because these shunted lambs are young, with only moderate vascular smooth muscle abnormalities, they displayed only moderate increases in pulmonary vascular resistance after CPB. The efficacy of ET_A receptor blockade in patients with more advanced pulmonary vascular disease warrants further investigation.

In summary, hypothermic CPB increased pulmonary vascular resistance, mean pulmonary arterial pressure, and plasma ET-1 concentrations in lambs with pre-

existing increased pulmonary blood flow and pulmonary hypertension. In addition, we found that pretreatment with a selective ET_A receptor antagonist completely blocked the increase in pulmonary vascular resistance and mean pulmonary arterial pressure after hypothermic CPB. These data suggest an important role for ET-1 and ET_A receptor activation in the pathophysiologic evidence of pulmonary hypertension after CPB and its associated increased pulmonary vascular reactivity. In addition to the alterations induced in the pulmonary circulation, ischemia to the brain and kidneys is a significant source of morbidity and death in children with congenital heart disease after hypothermic CPB.¹² ET-1 produces potent vasoconstriction in both the cerebral and renal circulations, and recent data demonstrate that treatment with ET_A receptor blockers decreases ischemic injury in animal models of cerebral and renal ischemia.²⁸⁻³⁰ Therefore the potential use of ET_A receptor blockers during CPB may have significant clinical benefits and warrants further study.

We thank Joan Keiser, PhD, for ongoing pharmaceutical expertise; Roger Chang, Rene Garrets, and Michael Johengen for technical assistance; and Lauren Gee, MPH, Senior Statistician, Department of Epidemiology & Biostatistics, University of California, San Francisco, for performing the statistical analysis.

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Discussion

Dr F. Mark Lupinetti (*Seattle, Wash*). This elegant study nicely convinces one of the validity of the investigators' hypotheses that ET-1 is an important mediator of pulmonary hypertension in this model and that ET_A receptor blockade effectively blunts this hemodynamic response. The elevations in pulmonary vascular resistance and pulmonary artery pressure though statistically significant were relatively modest. Could you speculate as to the possible influence of this finding on the thought regarding patients with more severe degrees of pulmonary hypertension? Do you believe that the patient with the markedly elevated pulmonary hypertensive crisis in the postoperative period has a quantitatively greater elaboration of ET-1, or is it a greater density of ET-1 receptors? Or perhaps should we be considering mediators other than ET-1 to explain the more clinically problematic situation?

Dr Petrossian. In the current study, both the control and the study lambs had a modest degree of pulmonary hypertension. In a previous study using this same model, the pulmonary artery pressure was up to 79% systemic. Patients with more advanced degrees of pulmonary hypertension are usually exposed to a longer period of increased pulmonary blood flow than the rather short (1-month) duration that the lambs in our experiment were exposed to. The severe degree of pulmonary hypertension in these patients is usually due to a combination of reversible and irreversible changes in the pulmonary vasculature.

Reversible pulmonary hypertensive disease in the setting of increased pulmonary blood flow is mediated by a complex interaction between nitric oxide and endothelin cascades. In the current study we have focused on the endothelin pathway alone. The mechanism of endothelin-mediated pulmonary hypertension is incompletely understood. There is considerable evidence, both clinically and experimentally, that endothelin gene expression is up-regulated in patients with advanced pulmonary hypertension. There is also evidence that ET_B receptors are involved in clearance of endothelin and that their expression is attenuated, thereby resulting in increased endothelin levels. Using our model, we have also shown (work currently in progress) that there is increased gene expression of ET_A receptors.

Dr Bradley Allen (*Chicago, Ill*). This is an elegant model. What happens in systemic pressures when you infuse PD156707? You told us what happened to the pulmonary pressures. Did you see any side effects in your systemic pres-

tures? I would expect CPB to cause a systemic inflammatory response, not to the lungs alone, but to the whole body. This can increase ET-1 expression, and I would have thought that you would also see an effect systemically.

Dr Petrossian. In response to your first question, we saw a mild and clinically insignificant drop in systemic arterial pressure after starting PD156707 and before instituting CPB. After bypass there was a significant drop in systemic pressures compared with levels before bypass, but this occurred to a similar extent in both the study lambs and the control lambs and was therefore not related to endothelin receptor blockade. The selec-

tive effect of PD156707 and the pulmonary rather than systemic artery pressure may be related to the more dense concentration of endothelin receptors on the pulmonary vasculature.

In response to your second question, we did not examine the lungs histologically, and we also did not assess endothelial cell function physiologically. We therefore cannot speculate on whether the favorable effects of PD 156707 on pulmonary artery pressures after bypass were mediated by protection of the lungs from the injurious effects of the inflammatory response accompanying CPB or by preservation of endothelial cell function.

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