Prevention of complement-induced pulmonary hypertension and improvement of right ventricular function by selective thromboxane receptor antagonism

The effect of complement activation on the pulmonary vascular system and on right ventricular function was studied in sheep ($n = 12$) by injection of cobra venom factor. Animals were instrumented for measurement of pulmonary flow, mean pulmonary artery pressure, right ventricular stroke work, arterial blood gases, and systemic vascular resistance. Blood was sampled from the left atrium and pulmonary artery to measure thromboxane B_2 , the metabolite of thromboxane A_2 , by radioimmunoassay. After baseline measurements, animals were randomly assigned to receive a selective thromboxane receptor antagonist SQ30741 as a 10 mg/kg bolus with an infusion of 10 mg/kg per hour or else to receive vehicle. Cobra venom factor was then injected (30 U/kg) in all animals, and data were recorded at 15, 30, 60, 90, and 120 minutes. In control animals there was a 2.4-fold increase in mean pulmonary artery pressure and a 76 % increase in right ventricular stroke work at 15 minutes from baseline ($p < 0.05$); these values remained elevated for 30 minutes and returned to baseline by 1 hour with no change in systemic vascular resistance. Arterial oxygenation decreased by 124 % at 15 minutes and remained depressed through the experiment, but in treated animals oxygen tension remained unchanged from baseline. Thromboxane B_2 increased 95 % from baseline in the control group and 1.5 fold in treated animals and followed a similar time course as the functional measurements ($p < 0.05$). A pulmonary vascular thromboxane B_2 gradient of approximately 1000 pg/ml was measured at 15 and 30 minutes in both control and treated groups. ($p < 0.05$) We conclude that after complement activation in this model pulmonary hypertension and decreased oxygen tension are mediated by thromboxane release from the pulmonary vascular bed. This increased afterload causes a stress on the right ventricle as demonstrated by the increased right ventricular stroke work. Selective thromboxane receptor antagonism may be a beneficial therapy for pulmonary hypertension in patients after cardiopulmonary bypass. (J THORAC CARDIOVASC SURG 1994;107:800-6)

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Despite advances in myocardial preservation and post-operative management, pulmonary hypertension remains

an important determinant of outcome after cardiac transplantation and mitral valve replacement.¹⁻⁴ The cause of acute right ventricular (RV) failure is most often the result of coupling the RV to an increased afterload from pulmonary hypertension, especially when it is not adapted to high pulmonary vascular resistance or when the RV has poor contractile reserve after prolonged preservation or arrest during cardiopulmonary bypass.⁵ Pulmonary hypertension with RV failure resulting from excessive alteration of pulmonary vascular resistance occurs as one of the effects of cardiopulmonary bypass.^{6,7}

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This acute reactive pulmonary hypertension may not respond to standard pulmonary vasodilator therapy but has been treated with prostacyclin or prostaglandin E₁ and left atrial phenylephrine (Neo-Synephrine) infusion. These results, however, are compromised by a concomitant reduction in systemic vascular resistance.⁸⁻¹²

After cardiopulmonary bypass, activation of complement and the production of thromboxane B₂ have been demonstrated in patients and in animal models.¹³⁻¹⁶ Exposure of the pulmonary vascular system to complement-activated serum has been demonstrated to produce pulmonary hypertension, hypoxia, and leukostasis.^{17, 18} In these studies, the pulmonary vascular and hypoxic responses were attenuated by pretreatment with cyclooxygenase synthetase inhibitors. Because thromboxane is a potent vasoconstrictor, we hypothesize that increased thromboxane production is a consequence of complement activation, causing acute pulmonary hypertension and hypoxia and compromised RV function. To test this hypothesis, we used a selective thromboxane receptor antagonist, SQ30741, in an animal model of complement-induced pulmonary hypertension by infusion of cobra venom factor (CVF).

Methods

Animal preparation. Adult Suffolk sheep weighing 30 to 35 kg were anesthetized intravenously with thiopental sodium (Pentothal) 15 mg/kg and their lungs were mechanically ventilated at a tidal volume of 15 ml/kg with an inspired oxygen fraction of 90%. A central venous catheter was placed percutaneously into the internal jugular vein, and the femoral artery was cannulated for arterial blood gas and pressure measurements. Maintenance anesthesia was controlled by intermittent administration of intravenous thiopental sodium (3 to 5 mg/kg). All animals received care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

The chest was opened via a median sternotomy and an electromagnetic flow probe (Carolina Medical Electronics, Inc., King, N.C.) was placed around the proximal pulmonary artery. Catheter-tipped high-fidelity micromanometers (Millar Instruments, Inc., Houston, Tex.) were introduced into the RV and left ventricular (LV) cavities and into the pulmonary artery. Piezoelectric crystals were tunneled into the septum, sutured to the RV epicardium, and connected to a sonomicrometer (Triton Technology, Inc., San Diego, Calif.) to measure RV septal free wall diameter. A volume conductance catheter (Webster Labs, Inc., Baldwin Park, Calif.) was introduced via the internal carotid artery, guided through the aortic root into the LV, and connected to a Leycom Sigma 5 DF signal processing unit (Cardiodynamics, Rijnsburg, The Netherlands). A vascular catheter was placed into the left atrium to sample blood and to monitor pressure. Physiologic data that were measured included heart rate, femoral artery pressure, pulmonary flow, pulmo-

nary pressure, LV pressure and volume, RV septal free wall diameter, RV pressure, and arterial blood gases.

Experimental protocol. After instrumentation, baseline physiologic measurements were recorded and blood samples were collected. Animals were randomized into two groups ($n = 6$) to receive either a selective thromboxane endoperoxide receptor antagonist, SQ30741 (Squibb Pharmaceuticals, Princeton, N.J.), or vehicle. The SQ30741 was prepared in a basic solution of 1N sodium hydroxide and titrated to a pH of 7.4 with an intravenous bolus of 10 mg/kg given over 3 minutes followed by an infusion of 10 mg/kg per minute.¹⁹ All animals were then given CVF 30 U/kg (Naje Haje; Diamedix, Miami, Fla.) intravenously to produce a model of acute pulmonary hypertension. CVF is a known activator of complement in sheep, and prior studies have documented dosing and activation of the complement system from CH₅₀ assay.²⁰ Data were recorded and samples collected at 15, 30, 60, 90, and 180 minutes after CVF injection.

Assays. Blood samples were collected simultaneously from the pulmonary artery and the left atrium in cold syringes containing aspirin and ethylenediaminetetraacetic acid and centrifuged at 2000 rpm for 15 minutes. The serum was withdrawn and placed in polyethylene tubes, stored at -90 ° C, and later thawed and analyzed for thromboxane A₂ with radioimmunoassay by detection of its metabolite thromboxane B₂. At the termination of the experiment a 5 gm section of the right lower lobe was collected, stored in a polystyrene tube at -90 ° C, and later assayed for myeloperoxidase.^{21, 22}

Data analysis. Results were summarized and compared by two-way analysis of variance with repeated measures applied to the time measurements. Least-squares estimated mean values generated by this two-way model are presented along with their standard errors. Differences were considered significant when $p < 0.05$. When either factor in the two-way model was significant, multiple comparisons were conducted by the Student-Newman-Keuls test.²³

Results

Hemodynamic values comparing control animals and animals treated with SQ30741 are summarized in Table I. After injection of CVF there was a significant rise from baseline in pulmonary vascular resistance, mean pulmonary artery pressure, and RV stroke work in all control animals. These values peaked at 15 minutes (Fig. 1, A), gradually began to decline at 30 minutes, and reached baseline again by 1 hour. Although not reaching statistical significance, an associated drop in pulmonary flow of 10% occurred at 15 minutes and a 20% decrease at 30 minutes. In animals receiving SQ30741 these changes were effectively prevented inasmuch as values from 15 to 120 minutes were not significantly changed from baseline (Fig. 1, B). Left atrial pressure was 0 to 1 mm Hg in both groups throughout the experiment.

Thromboxane B₂ levels increased in both control and treated groups (Table II) after a time course that paralleled the increase in pulmonary vascular resistance (Fig. 1, A). A significant thromboxane B₂ gradient was measured across the pulmonary vascular bed (left atrial-pulmonary arterial values) during the increased pulmonary

Table I. Hemodynamic measurements

	Baseline	15 min	30 min	60 min	90 min	120 min
PVR (dynes · sec · cm ⁻⁵)						
Control	430 ± 109	1030 ± 101*	912 ± 101*	562 ± 101	505 ± 101	442 ± 101
SQ30741	351 ± 101	299 ± 101	268 ± 101	290 ± 101	386 ± 101	395 ± 101
MPAP (mm Hg)						
Control	8.2 ± 1.7	19.4 ± 1.6*	17.8 ± 1.6*	10.1 ± 1.6	9.2 ± 1.6	9.4 ± 1.6
SQ30741	10.1 ± 1.6	8.6 ± 1.6	8.0 ± 1.6	7.5 ± 1.6	8.2 ± 1.6	9.2 ± 1.6
QPa (ml/min)						
Control	33.9 ± 4.8	29.6 ± 4.6	27.2 ± 4.6	25.3 ± 4.6	26.4 ± 4.6	28.0 ± 4.6
SQ30741	37.4 ± 4.6	40.4 ± 4.6	39.6 ± 4.6	35.4 ± 4.6	28.5 ± 4.6	30.9 ± 4.6
RVSW (dynes · cm)						
Control	266 ± 74	467 ± 70*	417 ± 70*	230 ± 70	233 ± 70	256 ± 70
SQ30741	349 ± 70	328 ± 71	302 ± 70	285 ± 70	262 ± 70	301 ± 70
RV dp/dt (mm/sec)						
Control	401 ± 75	600 ± 70	627 ± 70	445 ± 69	415 ± 70	402 ± 70
SQ30741	790 ± 69	677 ± 75	626 ± 82	509 ± 75	536 ± 83	545 ± 70
LV dp/dt (mm/sec)						
Control	1815 ± 234	1684 ± 222	1469 ± 222	1517 ± 222	1474 ± 222	1569 ± 222
SQ30741	1697 ± 199	1700 ± 208	1552 ± 221	1267 ± 207	1437 ± 221	1552 ± 199
LVSW (dynes · cm)						
Control	1097 ± 446	955 ± 545	580 ± 185	610 ± 320	740 ± 420	899 ± 448
SQ30741	1656 ± 1010	1976 ± 1492	1356 ± 1095	1194 ± 971	1309 ± 842	1172 ± 371
SVR (dynes · sec · cm ⁻⁵)						
Control	3019 ± 608	3140 ± 608	3513 ± 606	4300 ± 606	3785 ± 527	3678 ± 527
SQ30741	2711 ± 408	2420 ± 493	2605 ± 493	1871 ± 495*	2303 ± 495	3172 ± 408
HR (beats/sec)						
Control	113 ± 14	116 ± 13	112 ± 10	113 ± 12	110 ± 14	107 ± 7
SQ30741	110 ± 9	103 ± 5	103 ± 9	95 ± 8	100 ± 11	98 ± 6

Values are mean ± standard error. PVR, Pulmonary vascular resistance; MPAP, mean pulmonary artery pressure; QPa, pulmonary flow; RVSW, right ventricular stroke work; RV dp/dt, right ventricular pressure rate change; LV dp/dt, left ventricular pressure rate change; LVSW, left ventricular stroke work; SVR, systemic vascular resistance; HR, heart rate;

p < 0.05 compared with baseline.

Table II. Peripheral oxygen tension and serum thromboxane values

	Baseline	15 min	30 min	60 min	120 min
Po ₂ (mm Hg)					
Control	433 ± 32	193 ± 79	168 ± 64	206 ± 30	236 ± 28
SQ30741	494 ± 68	505 ± 62	496 ± 68	556 ± 44	509 ± 60
TXB ₂ (pg/ml)					
PA control	1728 ± 406	2824 ± 406	2392 ± 406	2025 ± 406	1930 ± 406
LA control	1877 ± 442	3588 ± 442	3187 ± 442	2130 ± 442	1891 ± 442
TXB ₂ (pg/ml)					
PA SQ30741	2087 ± 406	3781 ± 406	5695 ± 406	3194 ± 406	2920 ± 406
LA SQ30741	2033 ± 442	5175 ± 442	6638 ± 442	3079 ± 442	2778 ± 442

Values are mean ± standard error. Po₂, Peripheral arterial oxygen tension; TXB₂ thromboxane B₂; LA, left atrium; PA, pulmonary artery.

vasoconstriction, but thromboxane levels returned to baseline levels by 1 hour (Fig. 2). Arterial blood gas measurements demonstrate a persistent decrease in the oxygen tension at 15 minutes (Table II); by contrast, in the SQ30741 group, values remained near baseline levels (Fig. 1, A, and B). Systemic pH was maintained between 7.40 and 7.50 throughout the experiment by adjusting respiratory rate. Analysis of lung tissue with myeloperoxidase (units per milliliter per gram) showed no significant

difference between the two groups (control = 0.26 ± 0.022; SQ30741 = 0.22 ± 0.205).

Discussion

Our results demonstrate that after complement activation a transitory increase in mean pulmonary artery pressure, pulmonary vascular resistance, and RV stroke work occurs in association with hypoxia. These increases are completely prevented by prior administration of a

Fig. 1. Time course of pulmonary vascular resistance (*PVR*), right ventricular stroke work (*RVSW*), and arterial oxygenation (*PO2*) in control animals (**A**) and in SQ30741-treated animals (**B**) after injection of CVF at time 0. **p* < 0.05 as compared with baseline.

selective thromboxane receptor antagonist, SQ30741. Substantial production of thromboxane B₂ occurs across the pulmonary vascular bed, which parallels the physiologic changes and suggests that a significant por-

tion of the thromboxane B₂ may be produced by the pulmonary system. The complete prevention of these effects with thromboxane receptor antagonism indicates that pulmonary hypertension and hypoxia after

Fig. 2. Pulmonary vascular thromboxane B₂ gradient as calculated by subtraction of pulmonary artery values from left atrial values after CVF (30 μ /kg) injection at baseline. * $p < 0.05$ as compared with baseline.

complement activation are mediated by thromboxane.

These results confirm prior studies that have shown similar changes in pulmonary pressure and oxygenation to systemic complement activation or infusion of zymozan-activated plasma.²⁴⁻²⁶ Administration of cyclooxygenase synthetase inhibitors before infusion of zymozan-activated plasma has shown variable reductions in mean pulmonary artery pressure depending on the agent used.^{19, 27, 28} These reductions are probably the result of the nonspecificity of cyclooxygenase inhibition as an agent, but by using a selective receptor antagonist as a tool, we are able to determine more precisely the effects of thromboxane B₂.

In this model, the pulmonary vasculature responds to thromboxane by vasoconstriction, which increases pulmonary vascular resistance. Because these hearts had normal function before CVF injection, the control group was able to compensate for the increased afterload by increasing stroke work. It is probable that in a heart with poor reserve function or that had undergone a significant stress, such as cardioplegic arrest, the RV would not be able to compensate for the increased afterload and would fail.

The significant increase in the serum levels of thromboxane B₂ and development of a pulmonary vascular gradient suggest production within the pulmonary bed. Several authors have noted similar thromboxane B₂ gradients across the pulmonary vascular bed after complement activation and have concluded that the main source of thromboxane is the pulmonary system.^{24, 25} Perkowski

and associates¹⁸ have suggested that the source of thromboxane is lung endothelial or parenchymal cells, alveolar macrophages, or sequestered polymorphonuclear cells. In our results, SQ30741-treated animals had much higher serum thromboxane B₂ levels than control animals, although a similar left atrial-pulmonary arterial gradient was seen. One explanation may be that because receptor antagonism prevented the end organ response, a positive feedback loop was established that led to increased production. Another possible explanation is that because the receptor sites were nearly completely saturated, circulating serum levels increased.

The decrease in arterial oxygen tension was prevented in SQ30741-treated animals and appears to be primarily mediated by thromboxane. The mechanism of the decrease in arterial oxygen tension is probably related to the pulmonary vasoconstrictive effects of thromboxane producing pulmonary shunting and not to endothelial vascular damage. Although mild pulmonary endothelial injury has been observed after intravenous injection of zymozan-activated plasma, several authors have noted only mild changes in pulmonary lymph flow and changes in vascular permeability in short-term exposure to complement fragments.^{20, 27, 28}

One limitation of this study is that we were not able to measure the degree of complement activation in the two animal groups. However, complement activation has been assessed in a prior study using CVF in sheep, in which a higher dose (200 U/kg) was used with the goal to deplete the animals of complement.²⁰ We used a lower dose because patients' systems are not depleted of com-

plement from cardiopulmonary bypass but are at a lower level of activation.

Treatment of reactive postoperative pulmonary hypertension has consisted primarily of vasodilators, but because of their nonspecificity their effect on the peripheral vasculature limits their use. Use of prostaglandin E₁ and prostacyclin in these patients causes significant peripheral vasodilation, which sometimes requires the concurrent use of phenylephrine. Our results indicate that thromboxane receptor blockade with SQ30741 did not affect systemic vascular resistance. Prostacyclin and thromboxane have antagonizing actions and their balance may play a significant role in the modulation of pulmonary vascular tone.³⁰ It is possible that part of the effect of prostacyclin use in these patients may be the result of its effect on thromboxane metabolism or limiting the thromboxane-induced vasoconstriction effect by competitive inhibition.

Thromboxane receptor antagonism may be a useful therapy for reactive postoperative pulmonary hypertension and its detrimental effects on the RV and arterial oxygenation. This is a potentially useful adjunct to other vasodilatory therapy because it allows lower dosing with reduced systemic side effects.

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Discussion

Dr. Eric Mendeloff (*St. Louis, Mo.*). We presented similar data at the American College of Surgery meeting back in 1990 using a similar thromboxane receptor antagonist. We used a somewhat different model of pulmonary hypertension—protamine-induced reversal of heparin after bypass. We also induced a global ischemic injury with bypass. Our findings corroborated yours and we found that the thromboxane receptor antagonist, when given as a bolus after bypass, prevented the protamine reaction. We also found that with continuous infusion the global ischemic injury to the heart was also abolished.

Questions for you: Did you measure LV function and LV end-diastolic pressure? Do you think that impairment of LV function had any effect on the rise in pulmonary artery pressures? Did you measure myocardial oxygen consumption?

Dr. L. Henry Edmunds, Jr. (*Philadelphia, Pa.*). As far as I know, complement does not activate platelets to synthesize thromboxane A₂, which is measured as B₂, so I have no quibble with your results. I would be careful about ascribing thromboxane production to complement activation.

Dr. Smith. Dr. Mendeloff, we used CVF for this model and not cardiopulmonary bypass because we wanted to selectively look at complement-induced pulmonary hypertension without the multiple inflammatory products produced by cardiopulmonary bypass that have many systemic effects. We measured LV stroke work, LV end-diastolic pressure, and left atrial pressure in both groups, and we observed no statistically significant changes. The only physiologic effect produced by the CVF that we measured was an increase in pulmonary vascular resistance.

Dr. Edmunds, certainly the exact source of thromboxane production is not entirely known. In sheep, the platelets have been demonstrated not to produce thromboxane when stimulated in vitro. In our model the thromboxane is probably produced from the pulmonary vascular bed either from macrophages or from the leukocytes that are trapped in the lung tissue as a result of complement activation.