Effect of OKY 046, a thromboxane synthase inhibitor on lung vascular permeability after pulmonary embolism in sheep

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ABSTRACT OKY 046, a specific thromboxane synthase inhibitor, was used to investigate whether large pulmonary emboli, like microemboli, cause an increase in thromboxane A_2 and an associated increase in vascular permeability in sheep. Nineteen sheep were anaesthetised and had cannulas inserted into the afferent lymphatic of the caudal mediastinal lymph node and pulmonary and carotid arteries. Several days later the animals were pretreated with placebo or OKY 046 0.4 mg/kg one hour before being given clotted blood 0.5 g/kg intravenously. After embolisation in the control animals mean pulmonary artery pressure (MPAP) rose from 12 to 34 mm Hg and pulmonary artery wedge pressure (PAWP) fell from 4.4 to 1.5 mm Hg; the cardiac index did not change but the physiological shunt (Q_s/Q_t) rose from 17% to 50%. One hour after embolisation the platelet count fell from $76 \times 10^6/l$ whereas at 15 minutes thromboxane B_2 rose from 116 to 560 pg/ml in plasma and from 324 to 795 pg/ml in lymph ($p < 0.05$). By 2 hours the concentration of thromboxane B_2 was higher in lymph than in plasma. Lymph flow rose from 8.7 to a maximum of 27.3 ml/h at 15 minutes but despite the increase in flow the lymph:plasma (L:P) protein ratio did not fall, indicating an increased permeability of the blood vessels to protein. Pretreatment with OKY 046 inhibited the rise in plasma and lymph thromboxane B_2 , and limited the rise of Q_s/Q_t . The changes in MPAP, PAWP, cardiac index, platelet count, lymph flow, and L:P protein ratio, however, were no different from those in untreated sheep. These results indicate that a large pulmonary embolus leads to an increase in plasma and lung lymph thromboxane A_2 , which moderates the rise in Q_s/Q_t in part but not the increase in vascular permeability.

Several reports have documented an increase in microvascular permeability in the lungs after the intravenous infusion of various microemboli,¹ such as glass beads, air bubbles, barium sulphate, and platelets.²⁻⁴ The mechanism of the increase in permeability has been related to the activity of white blood cells⁵ and to thromboxane A_2 :⁶ leucopenia prevented the increase in permeability after air embolism,⁵ and inhibition of thromboxane synthase prevented the increase in permeability after thrombin infusion.⁶ A large pulmonary embolus causes platelet entrapment in the lungs and a rise in plasma

thromboxane B_2 , events similar to those accompanying microembolism produced by thrombin infusion.

This study tests the effect of a specific thromboxane synthesis inhibitor, OKY 046, on microvascular permeability and haemodynamic changes after the injection of large blood clots intravenously into sheep.

Methods

Experiments were conducted in 19 sheep weighing 39-45 kg. Animals were anaesthetised to allow cannulas to be inserted several days before pulmonary embolisation to avoid the effects of surgical trauma and anaesthesia at the time of embolisation. A cannula was inserted into the lymphatic channel draining into the caudal

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mediastinal lymph node by the technique described by Staub,⁷ a 14 gauge catheter was inserted into the jugular vein, a 7 French thermistor tipped catheter into the pulmonary artery (Instrumentation Laboratory, Lexington, Montana, USA), and a 20 gauge catheter into the carotid artery. On the day of the study 40 ml blood was withdrawn and 10 units thrombin/ml blood added. The clot was allowed to retract and after two hours the serum was decanted. The clot was cut into 3–5 mm cubes before intravenous infusion.

Measurements were conducted in awake sheep. Lymph was collected directly into a syringe from the cannula on the chest wall. A two hour collection was used to give a baseline lymph flow and protein ratio before embolisation. Measurements were taken at intervals up to four hours after embolisation. Strain gauge transducers (Bentley Laboratories, Irvine, California) were used to monitor the following pressures: mean pulmonary artery pressure, mean pulmonary artery wedge pressure, and mean arterial pressure. Cardiac output was measured in triplicate by thermodilution and divided by body weight to obtain the cardiac index. Blood gases, pH, haemoglobin, and oxygen saturation (Instrumentation Laboratory, models 282 and 812) were measured in pulmonary arterial and systemic arterial blood to calculate oxygen content in mixed venous, arterial, and pulmonary capillary blood. The Berggren equation was then used to calculate the percentage physiological shunt ($\dot{Q}_s/\dot{Q}_T$), where $\dot{Q}_s$ is blood flow leaving the lung with the same oxygen content as pulmonary arterial blood and $\dot{Q}_T$ is total flow.⁸ Blood and lymph samples were obtained before and 15, 30, 60, 120, and 240 minutes after embolisation.

Blood was collected in plastic syringes containing ethylene diamine tetra-acetic acid anticoagulant and aspirin, the later in a final concentration of 50 µg/ml. Plasma was immediately separated by centrifugation at 4°C, and then frozen at –20°C for subsequent assay of thromboxane B₂ and 6-keto-prostaglandin F_{1α} (PGF_{1α}). Aliquots of lymph were frozen for later prostanoid assay. All samples of plasma and lymph were coded and analysed blind. Radioimmunoassay for thromboxane B₂ and 6-keto-PGF_{1α}, the stable hydrolysis products of thromboxane A₂ and prostacyclin respectively, were conducted with an antibody supplied by Dr L Levine, Brandeis University, as reported elsewhere.⁹ The protein content of plasma and lymph was measured and the lymph: plasma protein ratio calculated.¹⁰ Circulating platelet and white blood cell counts were measured by phase microscopy.

After baseline measurements animals were divided randomly and nine received saline placebo and 10 the

imidazole derivative OKY 046 (kindly supplied by Ono Pharmaceutica, Osaka, Japan). This specific thromboxane synthase inhibitor was given in a dose of 0.4 mg/kg. One hour later a clot was introduced into the jugular venous catheter. At the end of the experiment all animals were anaesthetised and killed.

Statistical analysis to compare treated and control animals used analysis of variance and paired and non-paired *t* tests. Significance was accepted if $p < 0.05$. Results are reported as means with standard errors.

Results

In the saline pretreated sheep embolisation caused a rise in mean pulmonary artery pressure from 12 to 34 mm Hg at 15 minutes ($p < 0.05$), and a return to 26 mm Hg at 1 hour (fig 1). At 30 minutes mean central venous pressure had risen from 5.8 to 17.5 mm Hg ($p < 0.05$) and mean pulmonary artery wedge

Fig 1 Changes in mean pulmonary artery pressure (MPAP, mm Hg), cardiac index (CI, l min⁻¹ kg⁻¹), and heart rate (HR, beats/min) after embolisation in sheep pretreated with OKY 046 or placebo.

Fig 2 Change in physiological shunt ($\dot{Q}_s/\dot{Q}_T$) after embolisation in sheep pretreated with OKY 046 or placebo. * $p < 0.05$ for difference between groups.

pressure had fallen from 4.4 to 1.5 mm Hg ($p < 0.05$). The cardiac index did not vary from a baseline value of $0.1 \text{ l min}^{-1} \text{ kg}^{-1}$. Heart rate rose from 112 to 161 beats per minute at 15 minutes ($p < 0.05$) and mean arterial pressure rose insignificantly from a baseline value of 94 to 103 mm Hg. $\dot{Q}_s/\dot{Q}_T$ had risen at 15 minutes from 17% to 50% ($p < 0.001$; fig 2 and table).

The mean platelet count fell from 75.5 to $31.8 \times 10^6/\text{l}$ 15 minutes after embolisation ($p < 0.05$). There was a non-significant increase in

white blood cell count from a baseline value of $6 \times 10^6/\text{l}$ to $8 \times 10^6/\text{l}$ (table 1). Within 15 minutes of clot infusion there was an increase in plasma thromboxane B_2 from 116 to 560 pg/ml ($p < 0.01$) (fig 3) and in lymph thromboxane B_2 from 324 to 795 pg/ml ($p < 0.05$). Two hours after embolisation plasma thromboxane B_2 levels continued to rise (table 2). At 1 hour there was an increase in plasma 6-keto-PGF $_{1\alpha}$ concentration from 40 to 308 pg/ml ($p < 0.05$) and in lymph 6-keto-PGF $_{1\alpha}$ concentration from 210 to a peak of 1902 pg/ml (table 2).

After embolisation lymph flow rose from 8.7 to 27.3 ml/h at 15 minutes ($p < 0.001$), returning to 17.7 ml/h at 2 hours. The lymph-plasma protein ratio rose from a baseline value of 0.60 to 0.72 at 2 hours, though the change was not significant (fig 4). The fact that the lymph-plasma protein ratio did not fall indicates increased permeability of the lung vascular bed to protein and fluid. Pretreatment with OKY 046 prevented the rise of plasma and lymph thromboxane B_2 concentrations (fig 3) and moderated the rise in $\dot{Q}_s/\dot{Q}_T$ (fig 2). There were no differences in MPAP, PAWP, cardiac index, platelet counts, lymph flow, or lymph: plasma protein ratio between treated and control animals (figs 1 and 4).

Discussion

As anticipated, pulmonary embolism led to a rapid rise in MPAP and $\dot{Q}_s/\dot{Q}_T$ and a fall in platelet count. There was an increase in plasma and lymph thromboxane B_2 concentrations—though, surprisingly, lymph but not plasma thromboxane B_2 levels remained raised. The change in lymph thromboxane B_2 levels occurred over the same time as the rise in

Table 1 Baseline data obtained before embolisation

		Heart rate (/min)		MAP (mm Hg)		MPAP (mm Hg)		MPWP (mm Hg)		CVP (mm Hg)		Cardiac index (l min ⁻¹ kg)		$\dot{Q}_s/\dot{Q}_T$ (%)		Platelets ($\times 10^6/l$)		WBC ($\times 10^6/l$)		$\dot{Q}_L$ (ml/h)		L:P protein ratio	
Min		C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Mean	0	112	117	94	79	12	10	4.4	3.0	5.8	3.7	0.1	0.1	17	8.7	75.5	87	6.0	7.2	8.7	7.6	0.6	0.6
SEM		7.0	6.0	5.0	2.0	3.0	1.0	1.4	0.0	0.6	1.0	0.02	0.01	3.0	0.5	8.5	10	0.98	0.92	0.6	1.1	0.08	0.08
Mean	15	161	141	103	92	34	28	5.5	5.1	14	9.1	0.09	0.09	50	36	47.4	72	5.8	6.6	27	18.1	0.65	0.5
SEM		12	9.0	8.0	6.0	4.0	4.0	8.4	1.0	4.0	3.0	0.02	0.01	3.0	6.0	15	11	0.52	0.84	4.0	4.3	0.02	0.02
Mean	30	154	143	100	89	30	28	1.5	2.3	17.5	14	0.09	0.1	52	38	38	64	5.5	5.6	16	23.9	0.63	0.3
SEM		9.0	8.0	9.0	6.0	4.0	5.0	0.9	0.9	4.0	3.0	0.02	0.01	4.0	5.0	9.0	11	0.90	0.74	2.0	3.4	0.02	0.02
Mean	60	157	148	98	87	26	29	2.3	2.3	14.7	12.6	0.1	0.09	50	35	31.8	56	6.3	5.5	20	24.1	0.63	0.3
SEM		5.0	11	6.0	6.0	3.0	6.0	0.3	0.3	3.0	3.0	0.01	0.01	3.0	4.0	4.0	11	0.75	0.83	2.0	5.4	0.03	0.03
Mean	120	161	120	94	86	27	22	1.7	2.0	13	10.2	0.1	0.09	49	27	54	68	7.8	5.1	17.7	15.8	0.72	0.6
SEM		6.0	11	7.0	6.0	3.0	2.0	1.2	1.0	3.5	3.0	0.01	0.01	5.0	3.0	7.0	12	1.9	0.87	2.5	3.0	0.09	0.09
Mean	240	160	118	85	83	22	20	1.0	1.7	7.7	3.7	0.1	0.09	41	23	53	68	8.4	5.9	14.6	14.2	0.66	0.6
SEM		4.0	14	6.0	3.0	2.0	2.0	0.6	0.6	2.0	1.6	0.0	0.02	8.0	3.0	14	10	1.5	1.3	2.6	3.4	0.04	0.04

C—control; T—treated; MAP—mean arterial pressure; MPAP—mean pulmonary artery pressure; MPWP—mean pulmonary artery wedge pressure; CVP—central venous pressure; $\dot{Q}_s/\dot{Q}_T$ —physiological shunt ($\dot{Q}_s$ —bloodflow leaving the lung with the same oxygen content as pulmonary artery blood, $\dot{Q}_T$ —total flow); WBC—white blood cells; $\dot{Q}_L$ —lymph flow; L:P—lymph:plasma.

Fig 3 Plasma and lymph thromboxane B_2 concentrations after embolisation in sheep pretreated with OKY 046 or placebo. * $p < 0.05$.

physiological shunt (figs 2 and 3). The observation that OKY 046 prevented the increase in lymph and plasma thromboxane B_2 completely, but only partially limited the rise in physiological shunt, indicates that other agents besides thromboxane A_2 are responsible for the reduced ventilation-perfusion

Fig 4 Lymph flow and the lymph:plasma protein ratio before and after embolisation in sheep pretreated with OKY 046 or placebo.

ratio and hypoxaemia. Other studies have documented the importance of serotonin (5-hydroxytryptamine).¹¹

The observation that pulmonary embolism led to a twofold to threefold increase in lymph flow without a fall in the lymph-plasma protein ratio indicates an increase in the permeability of the vascular bed to fluids and proteins. An increase in hydrostatic pressures caused by partial vascular obstruction will induce an increase in fluid flow to the interstitium, but without a parallel increase in protein, so as the L:P protein ratio will fall.¹² The rise in thromboxane B_2 levels was not causally related to the increase in permeability since lymph flow and the lymph-plasma protein ratio were not altered when the synthesis of thromboxane A_2 was inhibited (fig 4). In other

Table 2 Mean (SD) plasma and lymph thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ (PGF $_{1\alpha}$) concentrations (pg/ml) before and up to four hours after embolisation

Time after embolisation (min)	Thromboxane B_2				6 keto-PGF $_{1\alpha}$			
	Plasma		Lymph		Plasma		Lymph	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
0	116 (30)	289 (107)	324 (66)	318 (55)	40 (70)	45 (60)	210 (89)	437 (55)
15	560 (84)	189 (42)	795 (266)	258 (33)	160 (50)	180 (90)	1010 (74)	537 (132)
30	524 (107)	236 (63)	838 (227)	247 (41)	200 (90)	300 (100)	1014 (212)	668 (139)
60	387 (59)	241 (92)	832 (66)	264 (38)	308 (355)	350 (150)	1902 (463)	810 (147)
120	284 (35)	212 (71)	1103 (234)	342 (80)	300 (250)	290 (200)	1900 (525)	1450 (140)
240	382 (143)	161 (76)	671 (156)	226 (62)	270 (220)	290 (150)	1200 (565)	1055 (307)

settings the inhibition of thromboxane synthase has limited microvascular permeability as in micro-embolism induced by thrombin infusion,⁶ white blood cell activation induced either by complement¹³ or by calcium ionophore,¹⁴ acid aspiration,¹⁵ and ischaemia.¹⁶ Pulmonary embolism must activate permeability pathways in addition to those related to thromboxane A₂. For example, platelets contain a high molecular weight agent that can induce permeability,¹⁷ or the interaction of an embolus with the lung could stimulate pulmonary mast cell synthesis of 5-lipoxygenase derived leukotrienes C₄, D₄, and E₄—potent permeability promoting agents.¹⁸ Finally, clot may stimulate alveolar macrophages to secrete platelet activating factor, another agent that provokes permeability.¹⁹

The ability of a pulmonary embolus to trigger pulmonary metabolic activity in which the arachidonic acid cascade plays a part is shown by the rise in lymph concentrations of thromboxane B₂ and 6-keto-PGF_{1α}. Presumably the major source of lymph 6-keto-PGF_{1α} is from endothelial cells stimulated by the thrombin clot.²⁰ The sustained rise in pulmonary lymph thromboxane B₂ concentrations while plasma thromboxane B₂ concentrations fall suggests a metabolic source in the lung parenchyma. Pulmonary synthesis of thromboxane A₂ is known to occur in other settings, such as anaphylactic reactions,²¹ where the stimuli gain access to the lungs via the vasculature as in embolisation. Several types of pulmonary parenchymal cells, including mast cells,¹⁸ fibroblasts,²² and endothelial cells,²³ have the capacity to synthesise thromboxane A₂. It remains possible, however, that the thromboxane source is related directly to platelets entrapped in the lungs, and that these cells secrete thromboxane B₂, which in turn diffuses into lung interstitium.

In summary, pulmonary embolism leads to an increase in microvascular permeability, which appears not to depend solely on thromboxane A₂ synthesis and mediation. This is in contrast to thrombin induced microembolisation, where the increase in permeability can be prevented by thromboxane inhibition.¹

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