

Selective Thromboxane A₂ Synthase Inhibition by OKY-046 Prevents Cardiopulmonary Dysfunction after Ovine Smoke Inhalation Injury

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Background: Because thromboxane A₂ is implicated in the pathophysiology of acute lung injury, the aim of this study was to evaluate the effects of selective thromboxane A₂ synthase inhibition on cardiopulmonary function in the experimental setting of severe smoke inhalation injury.

Methods: Sixteen adult sheep were operatively instrumented for chronic study. The injured intervention group was treated with the selective thromboxane A₂ synthase inhibitor OKY-046, whereas the injured control group received only the vehicle (n = 8 each).

Results: The progressive increase in thromboxane B₂ lung lymph concentrations in control animals was associated with increased transvascular fluid flux, augmented resistances in the pulmonary and systemic circulation, and a reciprocal decrease in cardiac output. In addition, end-systolic pressure–diameter relation and maximum +dp/dt were markedly depressed as compared with baseline (24 h: 14.3 ± 0.9 vs. 8.9 ± 0.5 mmHg/mm and 2,120 ± 50 vs. 1,915 ± 40 mmHg/s, respectively; each P < 0.05). Infusion of OKY-046 significantly inhibited pulmonary thromboxane B₂ delivery, attenuated the early increase in pulmonary vascular resistance, and blocked the increase in systemic vascular resistance. In addition, OKY-046 blunted and delayed the decrease in cardiac output and maintained end-systolic pressure–diameter relation, +dp/dt, and lung lymph flow at baseline values.

Conclusions: These findings suggest that selective thromboxane A₂ synthase inhibition may represent a goal-directed therapeutic approach to alleviate cardiovascular and pulmonary dysfunction in the setting of smoke inhalation injury.

CARDIOVASCULAR and pulmonary derangements after burn and smoke inhalation injuries are major contributors to morbidity and mortality in fire victims.¹ Annually, more than 75,000 fire victims are hospitalized in the United States, and more than 20% of this population experiences the consequences of smoke exposure.² Although new treatment strategies, including respiratory

support, adequate fluid challenge, and early surgical interventions, improved survival in burn patients, no specific therapy for the commonly associated smoke inhalation injury has yet been established.³

Because smoke inhalation injury continues to be the major cause of death in fire victims,⁴ we established an ovine model of acute lung injury^{5–8} that mimics the clinical situation of severe smoke inhalation injury and allows for the determination of myocardial contractility, pulmonary (dys)function, and microvascular permeability. We have previously demonstrated that cotton smoke exposure results in considerable pulmonary shunting,⁵ airway inflammation,⁶ and an augmented microvascular pressure gradient.⁷ Along with these changes, cardiac function is characteristically depressed, as indicated by reduced maximum elastance of left ventricular end-systolic pressure–volume relations and reduced maximal rate of change in left ventricular pressure (+dp/dt).⁸ In this context, it is noteworthy that smoke inhalation injury triggers the release of highly vasoconstrictive and bronchoconstrictive eicosanoids from the lung,^{9,10} which may aggravate systemic and pulmonary vasoconstriction and contribute to myocardial depression.¹¹

We have already reported that selective thromboxane inhibition improves cardiopulmonary performance in ovine endotoxemia^{12–14} and porcine burn sepsis.¹⁵ It has also been shown that thromboxane A₂ plays an important role in the pathogenesis of tumor necrosis factor α -induced pulmonary hypertension in sheep¹⁶ and that pretreatment with a thromboxane synthase inhibitor prevents pulmonary edema formation in a canine model of phorbol-ester-induced lung injury.¹⁷ In addition, Ishitsuka *et al.*¹⁸ have demonstrated that thromboxane A₂ is involved in the early phases of oleic acid-induced lung injury in guinea pigs and that rapidly acting thromboxane A₂ synthase inhibitors are effective in the prevention of acute lung injury.

We hypothesized that thromboxane B₂ and 6-keto-prostaglandin F_{1 α} , the stable degradation products of thromboxane A₂ and prostacyclin, respectively, play also a pivotal role in the pathogenesis of smoke inhalation injury. Therefore, the current study was designed as a prospective, controlled, randomized laboratory experiment to clarify whether specific thromboxane A₂ synthase inhibition prevents or at least ameliorates smoke inhalation injury–induced cardiovascular and pulmonary dysfunction in sheep.

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Materials and Methods

Subjects and Animal Care

The current study was performed in 16 adult sheep of the Merino breed with a mean body weight of 41.2 ± 1.2 kg and a body surface area of 1.01 ± 0.02 m². All experiments were undertaken in compliance with the guidelines of the National Institutes of Health and the American Physiologic Society for the care and use of laboratory animals and were approved by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston.

Animal Preparation

After a 12-h fasting period (with access only to water), sheep were operatively instrumented for chronic study during deep isoflurane anesthesia. Catheters (18 gauge, 36 in; Parke Davis, Sandy, UT) were placed in the thoracic aorta and the inferior vena cava *via* femoral arterial and venous cutdowns, respectively. In addition, a pulmonary artery catheter (Swan-Ganz, model 131F7; Baxter-Edwards Critical Care Division, Irvine, CA) was inserted into the right jugular vein through an 8.5-French percutaneous sheath introducer (Edwards Lifescience, Irvine, CA) and positioned in the pulmonary artery. For the cannulation of the caudal-mediastinal lymph node, a right-sided thoracotomy was performed at the level of the fifth intercostal space. The posterior end of the node was ligated by a modified technique originally described by Staub *et al.*¹⁹ In addition, a pneumatic occluder was placed around the inferior vena cava. Further, a left thoracotomy was performed at the level of the fifth intercostal space for implantation of pulse transit ultrasonic dimension transducers on the heart (LMT-53, 3-mm-diameter ceramic 5-MHz piezoelectric crystals; Crystal Biotech, Holliston, MA). Matched transducers were sutured to the anterior and posterior epicardium of the left ventricle for obtaining the maximum transverse external diameter in the plane of the minor axial circumference of the left ventricle. A silicon strain gauge pressure transducer (P5; Konigsberg Instrument, Pasadena, CA) was placed in the left ventricle *via* a stab incision through the apex cordis. This apical transducer, in conjunction with a subdermal electrode, allowed continuous electrocardiographic monitoring of the heart. The left atrium was also cannulated with a silicon tube (Duralastic Silicone Tubing DT08, 0.062-in ID, 0.125-in OD; Allied Biomedical, Paso Robles, CA) for the measurement of left atrial pressure. Because we have previously demonstrated that systemic areas may significantly contaminate the lymph drainage,²⁰ the right and left hemidiaphragms were cauterized to reduce systemic contamination of the lung lymphatic duct. Then, the chest was closed, and the incision was infiltrated with lidocaine to aid perioperative recovery. All catheters exited the thorax at a site distant from the incision. An infusion of lactated Ringer's solution

was started at $2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ to assure sufficient post-operative hydration. During the subsequent recovery period of approximately 7 days, the animals were individually housed in metabolic cages and had free access to food and water. Animals were only included in the study if their hemodynamics, blood gases, lymph flow, leukocyte count, and core body temperature were within normal ranges. One day before the experiment began, all catheters were connected to pressure transducers (Baxter-Edwards Critical Care) with continuous flushing devices (heparin-saline solution, 3 U/ml). At the day of the experiment, each animal received a tracheostomy (Shiley, 10-mm ID, 13.3-mm OD; Tyco Healthcare Group LP, Pleasanton, CA) and a urinary bladder catheter (Dover, 12–14 French; Sherwood Medical, St. Louis, MO) using ketamine and halothane as anesthetics.

Animal Grouping and Drug Infusion

After a baseline measurement in the healthy state, sheep were randomly allocated to either the injured control group (control, $n = 8$) or the injured intervention group (OKY, $n = 8$) being treated with the specific thromboxane A₂ synthase inhibitor OKY-046 (Ozagrel, 3-[4-(1H-imidazol-1ylmethyl)phenyl]-2E-propanoic acid; Ono Pharmaceutical Co., Osaka, Japan). In the latter group, a 10-mg bolus infusion of OKY-046 was given 10 min before the injury and was followed by a continuous infusion of $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ throughout the entire 24-h study period. The control group received only the vehicle (normal saline).

Experimental Protocol

All sheep were subjected to smoke inhalation injury according to an established protocol.^{5–8,21} Isoflurane (an ideal volatile anesthetic to maintain, but not to induce ovine anesthesia) was used for the operative procedures, whereas halothane (an inhalational anesthetic with a high analgesic potency) was administered during the induction of the injury. Ketamine was only used as a premedication to facilitate intubation. During the experiments, no anesthetics were administered, to avoid potential effects on the cardiopulmonary response. In brief, 4×12 breaths of cotton smoke ($< 40^\circ\text{C}$) were delivered *via* a modified bee smoker that was filled with 40 g burning cotton toweling and attached (*via* a modified endotracheal tube containing an indwelling thermistor from a Swan-Ganz catheter) to the tracheostomy tube. Arterial carboxyhemoglobin plasma concentrations were determined immediately after each set of smoke inhalation and served as an index of acute lung injury.^{5,21} After induction of the injury, anesthesia was discontinued, and the sheep were allowed to awaken.

Throughout the entire experiment, animals were equally mechanically ventilated (Servo Ventilator 900C; Siemens-Elcoma, Stockholm, Sweden). Because sheep have a higher pulmonary compliance than humans, they were ventilated with a tidal volume of 15 ml/kg. To

prevent atelectasis, a positive end-expiratory pressure of 5 cm H₂O was applied. During the first 3 h after injury, the inspiratory oxygen fraction was set at 100%, and the respiratory rate was set at 30 breaths/min to induce rapid carbon monoxide clearance. Ventilation was then adjusted to maintain sufficient oxygenation (arterial oxygen saturation > 95%, arterial oxygen tension > 90 mmHg) whenever possible.

All sheep were studied in the standing position while unanesthetized and undistressed. During this time, they were fluid resuscitated with lactated Ringer's solution (3 ml · kg⁻¹ · h⁻¹) and had free access to food from 1 h after injury.

Data Acquisition

The ultrasonic dimension transducers were connected to a Valpey-Fischer VF-1 Dimension System (Valpey-Fischer, Hopkinton, MA). The ultrasonic signal was amplified and continuously monitored using an oscilloscope (model 2213; Tektronix Inc., Beaverton, OR). Mean arterial pressure, mean pulmonary arterial pressure, and left atrial pressure were measured with Statham-Gould P23XL-1 transducers. The pressure transducers and the electrocardiogram were connected to a physiologic recorder (model OM9; Electronics for Medicine, Pleasantville, NY). The highest value of the left ventricular pressure was assumed to be the same as the peak aortic pressure; the lowest value was assumed to be 0 pressure (atmospheric pressure as reference). At the end of the study, these values were again checked, and it was certified that there was no drift over 24 h in each experiment. All dimensions, pressures, and electrocardiographic data were digitalized at 250 Hz with an RTI-800 analog to digital converter (Analog Devices Inc., Norwood, MA) and stored on magnetic media. Data were collected and averaged over one respiratory cycle, and at least five cardiac cycles were normalized to exclude the effect of respiration on the left ventricular dimension and pressure. Cardiac output was measured using the thermodilution technique with a Swan-Ganz catheter connected to a cardiac output computer (model 9520; Edwards Laboratory, Irvine, CA). Cardiac index, systemic vascular resistance index, and pulmonary vascular resistance index were calculated using standard equations. Lung lymph flow was measured with graduated test tubes and a stopwatch. Lymph and plasma protein concentrations were determined with a protometer (Refractometer; National Instruments, Baltimore, MD). Protein flux was calculated by multiplying lung lymph flow by the lymph to plasma protein concentration ratio. Heparinized samples of arterial plasma or lymph, collected with 2 µg/ml 1-phenyl-3-pyrazolidone, were immediately frozen at -80°C after collection and centrifugation. Subsequently, thromboxane B₂ and 6-keto-prostaglandin F_{1α} were measured by radioimmunoassay.²² The delivery of thromboxane B₂ to the lung was calculated by

multiplying lung lymph flow with the concentration of thromboxane B₂ in the lung lymph. In the same way, the delivery of 6-keto-prostaglandin F_{1α} to the lung was calculated. Plasma conjugated dienes, lipid peroxidation products, were measured spectrophotometrically (Spectronic 1001; Milton Roy Co., Houston, TX) at a wavelength of 233 nm.²³ Oxygen and carbon dioxide tensions in arterial and mixed venous blood were analyzed at 37°C and were corrected for core body temperature (System 1302; Instrumentation Laboratory, Lexington, MA). Oxygen saturation and carboxyhemoglobin concentrations were determined with an oximeter (CO-Oximeter 682; Instrumentation Laboratory).

Sonographic Measurements and Euthanasia of the Animals

An intraventricular pressure transducer and crystallographic dimension transducers were implanted for the determination of the end-systolic pressure-diameter relation. This relation is linear, unaltered by changes in preload or afterload, and sensitive to alterations in the inotropic state of the left ventricle.²⁴ The external minor axis was used in this analysis because it correlates closely with simultaneous left ventricular volumes under all conditions, during both diastole and systole.²⁵ End systole was defined as the point of maximum elasticity calculated by the ratio of instantaneous left ventricular pressure to instantaneous left ventricular diameter. In each group, the inferior vena cava was briefly occluded to reduce the preload of the left ventricle during determinations of the end-systolic pressure-diameter relation. Simultaneous left ventricular pressure and diameter loops were used to determine the end-systolic pressure-diameter relation of the left ventricle. The inferior vena cava occlusion was performed to minimize reflex sympathetic activity to the heart while measuring end-systolic pressure-diameter points for several curves. Without this approach, the end-systolic pressure-diameter maximum might have been changed. A line of best fit was described by the computer by the means of linear regression. The computer calculated the slope of the linear regression line and reported it as end-systolic pressure-diameter relation. Notably, only measurements with an *r* value greater than 0.9 were accepted.

After completion of the 24-h experiment, the animals were anesthetized with ketamine (15 mg/kg) and killed by intravenous injection of 60 ml saturated potassium chloride. Immediately after death, the proper positions of the ultrasonic dimension transducers were verified in every sheep.

Statistical Analysis

Data are presented as mean ± SEM. For statistical analysis, Sigma Stat 2.03 software (SPSS Inc., Chicago, IL) was used. Using a Kolmogorov-Smirnov test for goodness of fit to normal distribution, normality was obtained

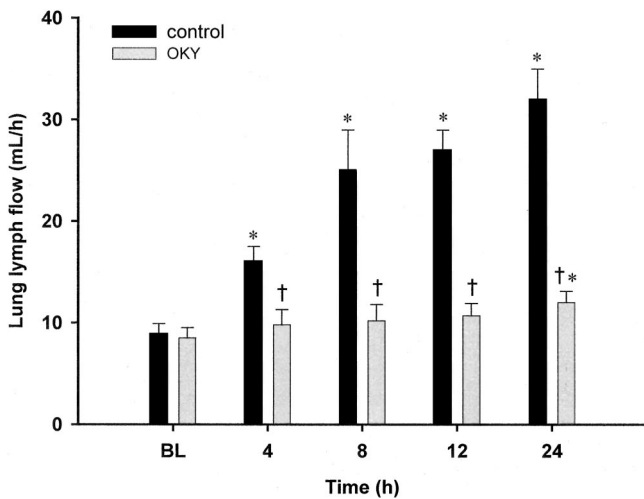


Fig. 1. Lung lymph flow of injured, untreated controls (control, $n = 8$) and injured sheep treated with the specific thromboxane A_2 synthase inhibitor OKY-046 (OKY, $n = 8$) at baseline (BL; 0 h) and 4, 8, 12, and 24 h after smoke inhalation injury. Data are expressed as mean \pm SEM. * $P < 0.05$ versus baseline. † $P < 0.05$ versus control.

for all measurements. A two-way analysis of variance for repeated measurements with appropriate Student-Newman-Keuls *post hoc* comparisons was used to calculate differences within and between groups. A P value of less than 0.05 was considered as statistically significant. The correlation coefficient r was determined using the Pearson product moment formula.

Results

The arterial carboxyhemoglobin plasma concentrations determined immediately after the fourth set of cotton smoke exposure averaged $65 \pm 5\%$ in the control group and $67 \pm 6\%$ in sheep treated with OKY-046, indicating that both groups were equally injured. With aggressive fluid resuscitation, all animals survived the 24-h study period.

Throughout the entire experiment, lung lymph flow was significantly higher in the control group as compared to the OKY group (fig. 1). Because there were no changes in the lymph to plasma protein concentration

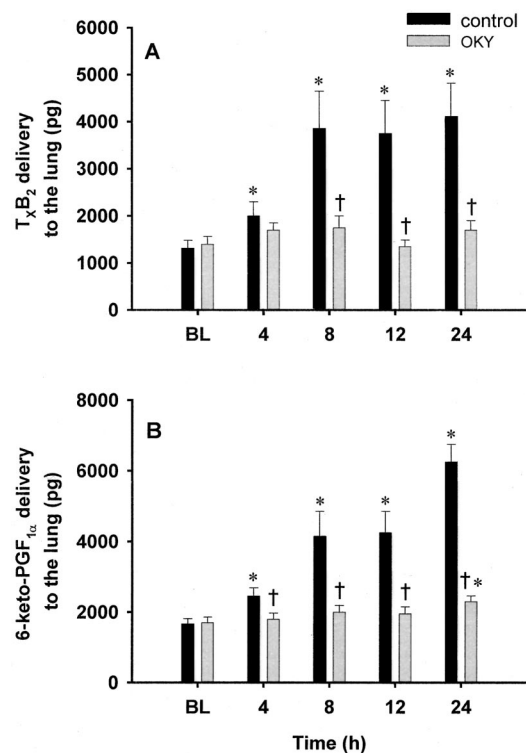


Fig. 2. (A) Delivery of thromboxane B_2 (T_xB_2) and (B) 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) to the lung in injured, untreated controls (control, $n = 8$) and injured sheep treated with the specific thromboxane A_2 synthase inhibitor OKY-046 (OKY, $n = 8$) at baseline (BL; 0 h) and at 4, 8, 12, and 24 h after smoke inhalation injury. Data are expressed as mean \pm SEM. * $P < 0.05$ versus baseline. † $P < 0.05$ versus control.

ratio in either group, the change in protein flux was similar to the changes in lung lymph flow (table 1).

There was a marked increase in thromboxane B_2 delivery to the lung in injured control animals, whereas pulmonary delivery of thromboxane B_2 remained unchanged in the OKY group (fig. 2A). Similarly, pulmonary delivery of 6-keto-prostaglandin $F_{1\alpha}$ progressively increased in the control group, but was unaffected in the OKY group from baseline to 12 h after injury (fig. 2B). Thromboxane B_2 plasma concentrations remained constant in either group, whereas there was an immediate and sustained increase in 6-keto-prostaglandin $F_{1\alpha}$ plasma concentration within each group but not between the two groups (fig. 3).

Table 1. Lung Permeability Variables

Variable	Group	Time after Injury				
		Baseline	4 h	8 h	12 h	24 h
L/P	Control	0.52 \pm 0.04	0.47 \pm 0.03	0.51 \pm 0.05	0.58 \pm 0.06	0.57 \pm 0.02
	OKY	0.52 \pm 0.05	0.48 \pm 0.07	0.49 \pm 0.08	0.55 \pm 0.06	0.56 \pm 0.04
PF	Control	4.3 \pm 0.5	7.3 \pm 1.0*	12.0 \pm 2.5*	15.0 \pm 2.3*	18.5 \pm 2.2*
	OKY	3.9 \pm 0.6	4.7 \pm 0.8†	5.5 \pm 1.2†	6.6 \pm 0.9†	7.0 \pm 0.6*†

Lymph-to-plasma protein concentration (L/P) and protein flux (PF), calculated by multiplying lymph flow with the lymph-to-plasma protein concentration (ml/h), in injured, untreated controls (control, $n = 8$), and after specific thromboxane A_2 synthase inhibition (OKY, $n = 8$) at baseline (before injury) and at 4, 8, 12, and 24 h after injury. Data are expressed as mean \pm SEM.

* $P < 0.05$ vs. baseline. † $P < 0.05$ vs. control.

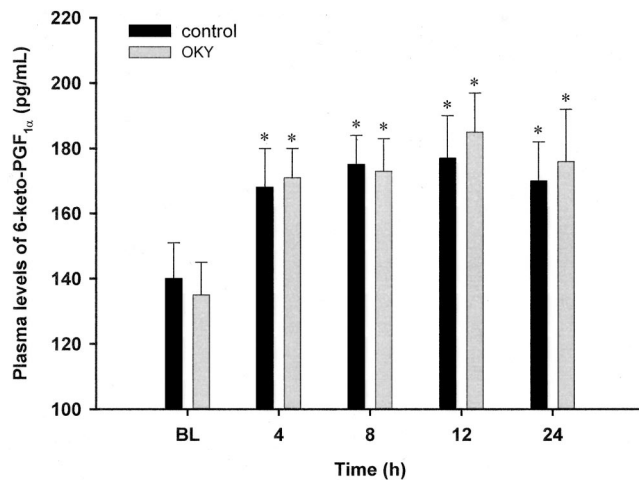


Fig. 3. Plasma concentrations of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) in injured, untreated controls (control, $n = 8$) and injured sheep treated with the specific thromboxane A_2 synthase inhibitor OKY-046 (OKY, $n = 8$) at baseline (BL; 0 h) and 4, 8, 12, and 24 h after smoke inhalation injury. Data are expressed as mean \pm SEM. * $P < 0.05$ versus baseline.

Hemodynamics of the systemic and pulmonary circulation are summarized in table 2 and show that the smoke-associated depression in cardiac index was attenuated and delayed in the OKY group. During the entire observation period mean arterial pressure, left atrial pressure and heart rate remained unchanged in either group. In the control group, however, there was a progressive increase in systemic vascular resistance index as compared to baseline. At 24 h after injury, systemic vascular resistance index was significantly higher in the control group than in the OKY group. Similarly, we noted successive increases in mean pulmonary arterial pressure and pulmonary vascular resistance index in the

control group that were significantly higher than in the OKY group (table 2).

The changes in left ventricular function are depicted in figure 4. Smoke inhalation injury contributed to an immediate and sustained reduction in the end-systolic pressure-diameter relation (control), whereas the end-systolic pressure-diameter relation remained unchanged in the intervention group (OKY). From 8 to 24 h after injury, the end-systolic pressure-diameter relation was significantly lower in the control group than in the OKY group. Likewise, left ventricular $+dp/dt$ remained unchanged in the OKY group. In the control group, maximum $+dp/dt$ was significantly depressed and lower than in the OKY group from 12 to 24 h after injury.

Compared with baseline, plasma conjugated dienes were significantly increased in the control group at 12 and 24 h after smoke exposure and were higher than in the OKY group. During the entire experiment, there were no changes in plasma conjugated dienes in the OKY group (fig. 5).

OKY infusion also attenuated the progressive increase in airway resistance observed in the control group, as indicated by decreases in peak and pause airway pressures (data not shown).

Discussion

Using an established and clinically relevant ovine model of smoke inhalation injury,^{5-8,21} we investigated the role of thromboxane A_2 in the pathogenesis of cardiopulmonary dysfunction. The intervention group was treated with OKY-046, a 1-alkyl imidazole derivative with an IC_{50} of 11 nM, acting as a specific thromboxane A_2 synthase inhibitor.^{26,27} The major finding was that

Table 2. Hemodynamics of the Systemic and Pulmonary Circulation

Variable	Group	Baseline	Time after Injury			
			4 h	8 h	12 h	24 h
CI, $l \cdot \text{min}^{-1} \cdot \text{m}^{-2}$	Control	6.5 \pm 0.3	5.8 \pm 0.3	5.4 \pm 0.4*	5.3 \pm 0.3*	4.6 \pm 0.2*
	OKY	6.2 \pm 0.2	5.9 \pm 0.2	6.1 \pm 0.4	5.6 \pm 0.3	5.3 \pm 0.1*†
HR, beats/min	Control	93 \pm 4	105 \pm 4	101 \pm 6	91 \pm 4	92 \pm 6
	OKY	91 \pm 3	94 \pm 4	92 \pm 4	88 \pm 3	89 \pm 4
MAP, mmHg	Control	89 \pm 3	90 \pm 3	86 \pm 3	89 \pm 3	85 \pm 3
	OKY	88 \pm 3	90 \pm 3	89 \pm 3	86 \pm 4	84 \pm 4
LAP, mmHg	Control	7 \pm 1	8 \pm 1	8 \pm 2	8 \pm 2	8 \pm 1
	OKY	8 \pm 1	9 \pm 1	8 \pm 1	7 \pm 1	7 \pm 1
SVRI, $\text{dyn/cm}^5 \cdot \text{m}^2$	Control	1,109 \pm 51	1,258 \pm 88	1,329 \pm 127	1,372 \pm 69*	1,503 \pm 70*
	OKY	1,124 \pm 27	1,234 \pm 44	1,191 \pm 66	1,256 \pm 76	1,256 \pm 68†
MPAP, mmHg	Control	19 \pm 1	25 \pm 1*	28 \pm 3*	27 \pm 2*	28 \pm 1*
	OKY	19 \pm 1	20 \pm 1†	20 \pm 1†	20 \pm 1†	21 \pm 1†
PVRI, $\text{dyn/cm}^5 \cdot \text{m}^2$	Control	155 \pm 10	230 \pm 13	302 \pm 32*	293 \pm 31*	346 \pm 46*
	OKY	146 \pm 3	155 \pm 10†	156 \pm 15†	179 \pm 6†	204 \pm 6†

Cardiopulmonary hemodynamics in injured, untreated controls (control, $n = 8$) and after specific thromboxane A_2 synthase inhibition (OKY, $n = 8$) at baseline (before injury) and at 4, 8, 12, and 24 h after injury. Data are expressed as mean \pm SEM.

* $P < 0.05$ vs. baseline. † $P < 0.05$ vs. control.

CI = cardiac index; HR = heart rate; LAP = left atrial pressure; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; PVRI = pulmonary vascular resistance index; SVRI = systemic vascular resistance index.

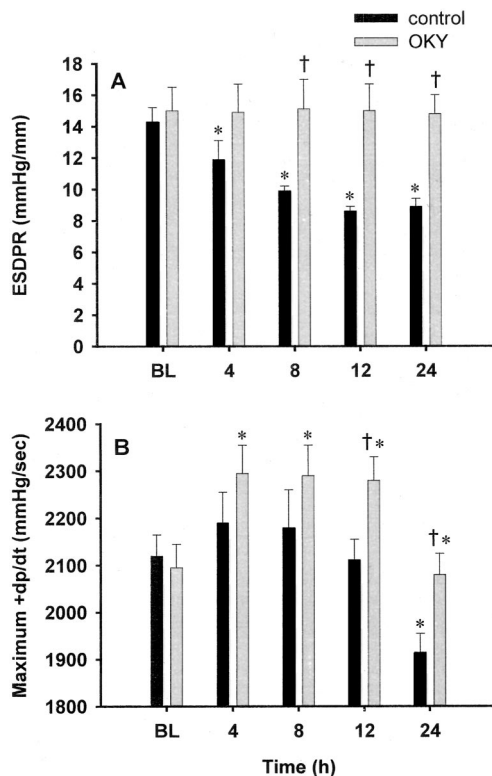


Fig. 4. (A) End-systolic pressure-diameter relation (ESDPR) and (B) maximum +dp/dt in injured, untreated controls (control, $n = 8$) and injured sheep treated with the specific thromboxane A_2 synthase inhibitor OKY-046 (OKY, $n = 8$) at baseline (BL; 0 h) and 4, 8, 12, and 24 h after smoke inhalation injury. Data are expressed as mean \pm SEM. * $P < 0.05$ versus baseline. † $P < 0.05$ versus control.

cotton smoke exposure increased lung lymph content of thromboxane B_2 (stable end product of thromboxane A_2), which in turn augmented transvascular fluid flux and contributed to significant decreases in both end-systolic

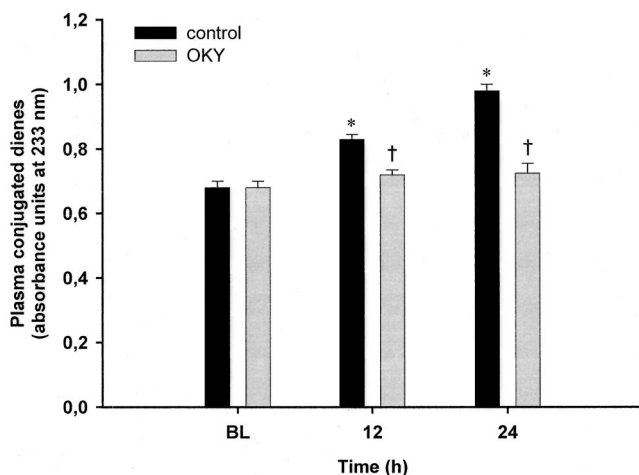


Fig. 5. Plasma concentrations of conjugated dienes in injured, untreated controls (control, $n = 8$) and injured sheep treated with the specific thromboxane A_2 synthase inhibitor OKY-046 (OKY, $n = 8$) at baseline (BL; 0 h) and 4, 8, 12, and 24 h after smoke inhalation injury. Data are expressed as mean \pm SEM. * $P < 0.05$ versus baseline. † $P < 0.05$ versus control.

pressure-diameter relation and maximum +dp/dt. Infusion of OKY-046 attenuated not only the increased pulmonary microvascular permeability seen after smoke inhalation injury, but also prevented myocardial depression.

The complex pathophysiology of smoke inhalation injury involves multiple factors, some well known and others still undetermined. In the past, we have demonstrated that cotton smoke exposure results in significant epithelial cell injury of the bronchial tree, which in turn contributes to the release of cytotoxic mediators, such as proteolytic enzymes, oxygen free radicals, and eicosanoids.^{6,28} The latter are synthesized by phospholipase A_2 , releasing arachidonic acid from membrane phospholipids. Subsequently, cyclooxygenase converts arachidonic acid to the precursor prostaglandin G_2 . Prostacyclin and thromboxane A_2 are downstream metabolites produced by prostacyclin synthase and thromboxane synthase, respectively. Prostacyclin is an abundant product of endothelial cells that causes vasodilation, whereas thromboxane A_2 promotes significant vasoconstriction.²⁹ Because recent studies in prostacyclin and thromboxane A_2 genetically deficient mice confirmed that the balance between those two eicosanoids modulates the cardiovascular response to injury,³⁰ we measured their stable end products, *i.e.*, prostaglandin $F_{1\alpha}$ and thromboxane B_2 .

The cardiopulmonary derangements in the untreated control group was linked to increased thromboxane B_2 delivery to the lung. Despite an augmented release of prostacyclin *via* the cyclooxygenase pathway, pulmonary vascular resistance index and mean pulmonary arterial pressure increased significantly over time. This finding is in full agreement with the study of Redl *et al.*,¹⁴ showing that increased prostaglandin $F_{1\alpha}$ plasma concentrations in endotoxemic sheep was ineffective to compensate for increased thromboxane B_2 concentrations and did not prevent pulmonary hypertension. Infusion of OKY-046, the specific thromboxane A_2 synthase inhibitor, attenuated the pulmonary vasoconstrictive response and improved right ventricular function, as indexed by improved ejection fraction and increased cardiac output. In the current study, infusion of OKY-046 prevented not only the increase in thromboxane B_2 but also in mean pulmonary arterial pressure and pulmonary vascular resistance index. This is of special clinical importance, because increased pulmonary resistance may lead to cardiac dysfunction/failure.³¹ The fact that thromboxane B_2 was only increased in the lung lymph but not in the plasma suggests that pulmonary inflammation triggers thromboxane synthesis in the airway. This postulation is supported by the finding of Takimoto *et al.*³² showing that endothelin 1, secreted from human bronchial epithelial cells, evokes the release of thromboxane B_2 in an autocrine manner. Similar results have also been reported by Janssens *et al.*³³ Using a sheep model of acrolein smoke-induced acute lung injury, the authors demonstrated that locally increased thrombox-

ane B₂ concentrations are implicated in the pathogenesis of pulmonary hypertension.

Reactive oxygen species are released in various forms of lung injury.³⁴⁻³⁶ In accord with previous studies,²³ we noted significantly increased plasma concentrations of conjugated dienes in response to the injury. Because conjugated dienes are lipid peroxidation products, their presence in the plasma is a direct evidence of an interaction of oxygen free radicals with lipid materials in the sheep. Because inhibition of thromboxane A₂ synthesis prevented the formation of conjugated dienes, it is most likely that thromboxane A₂ stimulated reactive oxygen species formation. This hypothesis is supported by the study of Paterson *et al.*,³⁷ demonstrating that there is a direct relation between the plasma concentrations of thromboxane B₂ 5 min after reperfusion and the magnitude of the subsequent intracellular H₂O₂ production. Furthermore, these authors reported that activated polymorphonuclear cells, in turn, lead to more thromboxane synthesis, a positive feedback mechanism resulting in further neutrophil activation. In this regard, it is especially important that neutrophils represent a main source of thromboxane release after acute lung injury.³⁸ Moreover, it must be considered that polymorphonuclear cells play a major role in the development of the primary lung injury associated with ovine smoke inhalation injury and may contribute to its systemic manifestation.³⁹ Therefore, it is most likely that OKY-046 limited neutrophil accumulation in the lung, an assumption that is supported by the study of Goldman *et al.*⁴⁰ Using a rat model of acid aspiration, the authors demonstrated that OKY-046 limited adhesion of polymorphonuclear cells.

The most significant finding of the current study was that the intrinsic myocardial contractility was not depressed to the same extent in the treated animals as in the control group. With inhalation injury, maximum elastance of left ventricle end-systolic pressure-volume relations decreased by more than 50%. When the question arises as to whether carbon monoxide exposure, *per se*, may have accounted for the decrease in myocardial contractility, it is noteworthy that we have previously demonstrated that smoke-associated myocardial depression is not linked to carbon monoxide intoxication; the exact mechanism, however, remained undetermined.⁸ When viewing the above-discussed studies together with the results of the present investigation, it seems that thromboxane B₂-mediated myocardial dysfunction was triggered by neutrophil accumulation in the injured lung. This hypothesis is supported by the study of Evangelista *et al.*,³⁸ reporting that acute lung inflammation is associated with infiltration of polymorphonuclear cells and contributes to thromboxane A₂-mediated myocardial ischemia in a rabbit model.

Ovine smoke inhalation injury was also associated with a marked increase in lung lymph flow, similarly to what has been previously observed in our model.³⁹ We noted

that administration of OKY-046 markedly blunted this response. Likewise, Enkhbaatar *et al.*⁴¹ demonstrated that the cyclooxygenase inhibitor ketorolac reduces pulmonary edema after combined burn and smoke inhalation injury, in part *via* inhibiting nitric oxide synthesis. These findings suggest that eicosanoid overproduction and the nitric oxide pathway are linked in the pathophysiology of smoke inhalation injury. However, future studies are needed to address this issue in greater detail.

This study has some limitations that we want to acknowledge. First, we cannot assure that exogenous administration of OKY-046 produces identical responses in sheep and patients when used under similar conditions. Because we did not investigate regional blood flow, it also remains undetermined how thromboxane A₂ synthase inhibition affected gastrointestinal blood flow in the current study. However, Sakurai *et al.*⁴² previously reported an early reduction in mesenteric blood flow in sheep with burn and smoke inhalation injury that corresponded to the period of reduced systemic blood flow. Because OKY-046 prevented the increase in systemic vascular resistance index and alleviated the decrease in cardiac index, it is likely that mesenteric blood was also improved by thromboxane inhibition. This notion is reinforced by the study of Tokyay *et al.*,⁴³ demonstrating that OKY-046 improved postburn mesenteric blood flow and decreased the rate of bacterial translocation in a porcine model of burn injury.

Although OKY-046 may prove therapeutic, we want to emphasize that the timing of administration may be critical, especially given the often disappointing clinical responses to other inhibitors in past trials in the critically ill. In this regard, it is also noteworthy that Ishitsuka *et al.*¹⁸ reported that a pretreatment with OKY-046 was more effective than a posttreatment of guinea pigs with acute respiratory distress syndrome. Therefore, future studies are needed to test the efficacy of this specific thromboxane A₂ inhibitor when it is given after smoke inhalation injury.

Notably, we have already demonstrated that ovine smoke inhalation injury is not only associated with increased thromboxane production, but also with excessive nitric oxide synthesis.⁴⁴ Because inhibition of each product only attenuated cardiopulmonary dysfunction but did not block the underlying pathogenesis, future studies are needed to determine whether a combination therapy of selective nitric oxide and thromboxane A₂ synthase inhibitors are useful to further reduce the degree of injury.

Conclusions

In this prospective study, we showed that thromboxane A₂ release secondary to smoke inhalation contributes to myocardial depression and increased pulmonary

microvascular fluid flux. Because OKY-046 markedly attenuated cardiopulmonary derangements and lipid peroxidation, specific thromboxane A₂ synthase inhibition seems to be a promising, goal-directed therapeutic target in the setting of smoke inhalation injury. Future studies investigating the role of thromboxane A₂ in the setting of acute respiratory distress syndrome are warranted and may help to test preventative measures and therapeutic strategies.

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References

- Shirani KZ, Pruitt BAJ, Mason ADJ: The influence of inhalation injury and pneumonia on burn mortality. *Ann Surg* 1987; 205:82-7
- Pruitt BAJ Jr, Goodwin CW, Mason ADJ Jr: Epidemiological, demographic and outcome characteristics of burn injury, *Total Burn Care*. Edited by Herndon DN. London, Edinburgh, New York, Philadelphia, St. Louis, Sydney, Toronto, WB Saunders, 2002, pp 16-32
- Sheridan R: Specific therapies for inhalation injury. *Crit Care Med* 2002; 30:718-9
- Tredget EE, Shankowsky HA, Taerum TV, Moysa GL, Alton JDM: The role of inhalation injury in burn trauma. *Ann Surg* 1990; 212:720-7
- Westphal M, Morita N, Enkhbaatar P, Murakami K, Traber L, Traber DL: Carboxyhemoglobin formation following smoke inhalation injury in sheep is interrelated with pulmonary shunt fraction. *Biochem Biophys Res Commun* 2003; 311:754-8
- Cox RA, Soejima K, Burke AS, Traber LD, Herndon DN, Schmalstieg FC, Traber DL, Hawkins HK: Enhanced pulmonary expression of endothelin-1 in an ovine model of smoke inhalation injury. *J Burn Care Rehabil* 2001; 22:375-83
- Isago T, Fujioka K, Traber LD, Herndon DN, Traber DL: Derived pulmonary capillary pressure changes after smoke inhalation in sheep. *Crit Care Med* 1991; 19:1407-13
- Sugi K, Theissen JL, Traber LD, Herndon DN, Traber DL: Impact of carbon monoxide on cardiopulmonary dysfunction after smoke inhalation injury. *Circ Res* 1990; 66:69-75
- Wang S, Lantz RC, Robledo RF, Brededa V, Hays AM, Witten ML: Early alterations of lung injury following acute smoke exposure and 21-aminosteroid treatment. *Toxicol Pathol* 1999; 27:334-41
- Quinn DA, Robinson D, Jung W, Hales CA: Role of sulfidopeptide leukotrienes in synthetic smoke inhalation injury in sheep. *J Appl Physiol* 1990; 68:1962-9
- Lefter AM: Interaction between myocardial depressant factor and vasoactive mediators with ischemia and shock. *Am J Physiol* 1987; 252:R193-205
- Fujioka K, Sugi K, Traber LD, Herndon DN, Traber DL: The effect of thromboxane synthase inhibition on cardiopulmonary function during endotoxemia in sheep. *J Burn Care Rehabil* 1990; 11:531-7
- Fujioka K, Sugi K, Isago T, Flynn JT, Traber LD, Herndon DN, Traber DL: Thromboxane synthase inhibition and cardiopulmonary function during endotoxemia in sheep. *J Appl Physiol* 1991; 71:1376-81
- Redl G, Abdi S, Traber LD, Nichols RJ, Flynn JT, Herndon D, Traber DL: Inhibition of thromboxane synthesis reduces endotoxin-induced right ventricular failure in sheep. *Crit Care Med* 1991; 19:1294-302
- Iglesias G, Zeigler ST, Lentz CW, Traber DL, Herndon DN: Thromboxane synthase inhibition and thromboxane receptor blockade preserve pulmonary and circulatory function in a porcine burn sepsis model. *J Am Coll Surg* 1994; 179:187-92
- Koizumi T, Kubo K, Kobayashi T, Sekiguchi M: Effects of thromboxane synthase inhibition on tumor necrosis factor-induced lung injury in sheep. *J Appl Physiol* 1992; 73:618-24
- Sprague RS, Stephenson AH, Lonigro AJ: OKY-046 prevents increases in LTb4 and pulmonary edema in phorbol ester-induced lung injury in dogs. *J Appl Physiol* 1992; 73:2493-8
- Ishitsuka Y, Moriuchi H, Hatamoto K, Yang C, Takase J, Golbidi S, Irikura M, Irie T: Involvement of thromboxane A₂ (TXA₂) in the early stages of oleic acid-induced lung injury and the preventive effect of ozagrel, a TXA₂ synthase inhibitor, in guinea-pigs: *J Pharm Pharmacol* 2004; 56:513-20
- Staub NC, Bland RD, Brigham KL, Demling R, Erdmann AJL, Woolverton CW: Preparation of chronic lung lymph fistulas in sheep: *J Surg Res* 1975; 19:315-20
- Drake R, Adair T, Traber D, Gabel J: Contamination of caudal mediastinal node efferent lymph in sheep. *Am J Physiol* 1981; 241:H354-7
- Westphal M, Morita N, Enkhbaatar P, Murakami K, Traber L, Traber DL: Acute effects of combined burn and smoke inhalation injury on carboxyhemoglobin formation, tissue oxygenation and cardiac performance. *Biochem Biophys Res Commun* 2004; 317:945-9
- Agnoli GC, Borgatti R, Cacciari M, Lenzi P, Marinelli M, Stipo L: Renal function and urinary prostanoid excretions in salt-depleted women: Comparative effects of enalapril and indomethacin treatment. *Prostaglandins Leukot Essent Fatty Acids* 1999; 60:87-9
- Chandra A, Katahira J, Schmalstieg FC, Murakami K, Enkhbaatar P, Cox RA, Hawkins HK, Traber LD, Herndon DN, Traber DL: P-selectin blockade fails to improve acute lung injury in sheep. *Clin Sci (Lond)* 2003; 104:313-21
- Williams RV, Lorenz JN, Witt SA, Hellard DT, Khoury PR, Kimball TR: End-systolic stress-velocity and pressure-dimension relationships by transthoracic echocardiography in mice. *Am J Physiol* 1998; 271:H1828-35
- Olsen CO, Rankin JS, Arentzen CE, Ring WS, McHale PA, Anderson RW: The deformational characteristics of the left ventricle in the conscious dog. *Circ Res* 1981; 49:843-55
- Ichikawa K, Tazawa S, Hamano S, Kojima M, Hiraku S: Effect of ozagrel on locomotor and motor coordination after transient cerebral ischemia in experimental animal models. *Pharmacology* 1999; 59:257-65
- Press JB, Wright WB Jr, Chan PS, Marsico JW, Haug MF, Tauber J, Tomcufcik AS: Thromboxane synthesis inhibitors and antihypertensive agents: 2-N-[(1H-imidazol-1-yl)alkyl]-1H-isoindole-1,3(2H)-diones and N-[(1H-1,2,4-triazol-1-yl)alkyl]-1H-isoindole-1,3(2H)-diones as unique antihypertensive agents. *J Med Chem* 1986; 29:816-9
- Herndon D, Traber LD, Linares H, Flynn JD, Niehaus G, Kramer G, Traber DL: Etiology of the pulmonary pathophysiology associated with inhalation injury. *Resuscitation* 1986; 14:43-59
- Narumiya S, Sugimoto Y, Ushikubi F: Prostanoid receptors: Structures, properties, and functions. *Physiol Rev* 1999; 79:1193-226
- Cheng Y, Austin SC, Rocca B, Koller BH, Coffmann TM, Grotzer T, Lawson JA, Fitzgerald GA: Role of prostacyclin in the cardiovascular response to thromboxane A₂. *Science* 2002; 296:539-41
- Vieillard-Baron A, Schmitt JM, Augarde R, Fellahi JL, Prin S, Page B, Beauchet A, Jardin F: Acute cor pulmonale in acute respiratory distress syndrome submitted to protective ventilation: Incidence, clinical implications, and prognosis. *Crit Care Med* 2001; 29:1551-5
- Takimoto M, Oda K, Sasaki Y, Okada T: Endothelin-A receptor-mediated secretion via autocrine and deoxyribonucleic acid synthesis via paracrine signaling in human bronchial epithelial cells. *Endocrinology* 1996; 137:4542-50
- Janssens SP, Musto SW, Hutchinson WG, Spence C, Witten M, Jung W, Hales CA: Cyclooxygenase and lipooxygenase inhibition by BW-755C reduces acrolein smoke-induced acute lung injury. *J Appl Physiol* 1994; 77:888-95
- Li C, Wright MM, Jackson RM: Reactive species mediated injury of human lung epithelial cells after hypoxia-reoxygenation. *Exp Lung Res* 2002; 28:373-89
- Morio LA, Hooper KA, Brittingham J, Li TH, Gordon RE, Turpin BJ, Laskin DL: Tissue injury following inhalation of fine particulate matter and hydrogen peroxide is associated with altered production of inflammatory mediators and antioxidants by alveolar macrophages. *Toxicol Appl Pharmacol* 2001; 177:188-99
- Weinberger B, Fakhzadeh L, Heck DE, Laskin JD, Gardner CR, Laskin DL: Inhaled nitric oxide primes lung macrophages to produce reactive oxygen and nitrogen intermediates. *Am J Respir Crit Care Med* 1998; 158:931-8
- Paterson IS, Klausner JM, Goldman G, Kobzik L, Welbourn R, Valeri CR, Shepro D, Hechtman HB: Thromboxane mediates the ischemia-induced neutrophil oxidative burst. *Surgery* 1989; 106:224-9
- Evangelista V, Dell'Elba G, Celardo A, Manarini S, Cerletti C: TXA₂-mediated myocardial ischemia as a consequence of an acute inflammatory reaction in the rabbit. *J Thromb Haemost* 2003; 1:314-9
- Schenarts PJ, Schmalstieg FC, Hawkins H, Bone HG, Traber LD, Traber DL: Effects of an L-selectin antibody on the pulmonary and systemic manifestation of severe smoke inhalation injuries in sheep. *J Burn Care Rehabil* 2000; 21:229-40
- Goldman G, Welbourn R, Kobzik L, Valeri CR, Shepro D, Hechtman HB: Synergism between leukotriene B₄ and thromboxane A₂ in mediating acid-aspiration injury. *Surgery* 1992; 111:55-61
- Enkhbaatar P, Murakami K, Shimoda K, Salisbury J, Cox R, Hawkins H, Traber L, Herndon D, Traber D: Ketorolac attenuates cardiopulmonary derangements in sheep with combined burn and smoke inhalation injury. *Clin Sci (Lond)* 2003; 105:621-8
- Sakurai H, Traber LD, Traber DL: Altered systemic blood flow after combined injury with burn and smoke inhalation. *Shock* 1998; 9:369-74
- Tokuyay R, Loick HM, Traber DL, Heggors JP, Herndon DN: Effects of thromboxane synthase inhibition on postburn mesenteric vascular resistance and the rate of bacterial translocation in a chronic porcine model. *Surg Gynecol Obstet* 1992; 174:125-32
- Soejima K, McGuire R, Synder N IV, Uchida T, Szabo C, Salzmann A, Traber LD, Traber DL: The effect of inducible nitric oxide synthase (iNOS) inhibition on smoke inhalation injury in sheep. *Shock* 2000; 13:261-6