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Halothane-Oxidant Interactions in the Ex Vivo *Perfused Rabbit Lung*

Fluid Conductance and Eicosanoid Production

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Background: **The present studies were undertaken to determine the interactions between halothane and oxidative injury with respect to endothelial integrity, as measured by pulmonary capillary filtration coefficient (K_{fc}), and production of arachidonic acid-derived mediators, in perfused rabbit lungs challenged with the oxidant *tert*-butyl-hydroperoxide (*t*-bu-OOH).**

Methods: **Isolated lungs were prepared from 27 New Zealand white rabbits (2–3 kg) and were perfused with Ca^{2+}-free Krebs-Henseleit buffer solution. In group A (n = 9), lungs were ventilated with halothane 2.5% in carrier gas (5% CO_2 in air); in group B (n = 9), with carrier gas alone; and in group C (n = 9), with carrier gas, but without injury. The lungs in the two injury groups (A and B) received four infusions of *t*-bu-OOH, 200 μM, over 1 min, directly into the pulmonary artery. The uninjured lungs received four infusions of vehicle (normal saline). K_{fc} was determined after each *t*-bu-OOH infusion. Concentrations of thromboxane B_2 (TxB_2) and 6-keto-prostaglandin $F_{1\alpha}$ were measured in samples of effluent perfusate obtained before and 30 s after the end of each infusion of *t*-bu-OOH. The wet/dry weight ratio of each pair of lungs was determined at the end of each experiment.**

Results: **K_{fc} progressively increased after each infusion of oxidant in group A when compared with the other two groups. Lung wet/dry ratios were elevated in group A (14.3 ± 0.7) and group B (13.2 ± 0.2) compared with group C (12.1 ± 1.1). TxB_2 production in group A (2206 ± 263 $pg \cdot min^{-1} \cdot g^{-1}$ dry lung tissue) was greater than in group B (1413 ± 127) by the final infusion of *t*-bu-OOH.**

Conclusions: ***Ex vivo*** **perfused rabbit lungs ventilated with halothane exhibited, simultaneously, evidence of greater fluid conductance across the pulmonary capillary bed and production of thromboxane A_2 when challenged with oxidant than did lungs ventilated with carrier gas. Both of these effects may be mediated by halothane-related enhancement of intracellular endothelial Ca^{2+} mobilization stimulated by intrapulmonary infusion of oxidant. (Key words: Anesthetics, volatile: halothane. Lung metabolism. Prostaglandins: 6-keto-prostaglandin F_{1a}; thromboxane B_2. Respiratory distress syndrome, adult. Species: rabbit.)**

VOLATILE anesthetics may modulate the intensity of lung inflammatory injury of both infectious and noninfectious origins. Recent studies have demonstrated: (1) decreased pulmonary vascular endothelial resistance to injury by activated neutrophils in the presence of halothane and isoflurane,[1] (2) increased alveolar-capillary permeability after acid aspiration lung injury in rats exposed to halothane or isoflurane,[2] and (3) altered lung pathology in mice infected with influenza A virus and exposed to either halothane or isoflurane.[3] Inhalation anesthetics also enhance the pressor effect of the organic oxidant *tert*-butyl-hydroperoxide (*t*-bu-OOH) in the *ex vivo* perfused rabbit lung.[4] The enhanced pressor response is accompanied by augmented recovery of thromboxane A_2 (TxA_2) from pulmonary venous effluent after challenge with *t*-bu-OOH. This effect may occur because the highly lipid-soluble inhalational anesthetic agents displace cell membrane phospholipids, making them more available for hydrolysis.[5] Arachidonic acid metabolites may act as markers of lung injury and may aid in lung adaptation to injury.[6,7] Substances that inhibit the production of arachidonic acid metabolites or inhibit activation of arachidonic acid metabolic pathways may result in modulation of physiologic lung responses to injury or perturbation,[8–11] yet the contribution of arachidonate

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metabolites to the process of injury itself remains unclear and varies from species to species.[7]

Infusion of *t*-bu-OOH into the *ex vivo* perfused lung induces diffuse injury and, subsequently, high permeability pulmonary edema in an experimental model of adult respiratory distress syndrome.[4,12] The oxidant *t*-bu-OOH may produce injury by initiation of lipid peroxidation chain reactions within cell membranes.[13] Although readily scavenged by glutathione peroxidase, *t*-bu-OOH is highly lipid-soluble and may interact in the hydrophobic regions of cell membranes with Fe^{2+} and Fe^{3+} complexes to initiate lipid peroxidation and fatty acid chain lengthening and branching.[13] Such oxidant-mediated alterations in membrane lipid structure eventually may change membrane properties, such as fluidity, or the functions of proteins, such as receptors and ion channels, which are intercalated in the membrane structure and usually act as lipid chain interrupters, helping to maintain membrane fluidity.[13]

Because of the previously demonstrated interactions between inhalational anesthetics and lung inflammatory injury,[1-4] we undertook the following experiments to determine whether halothane will change the susceptibility of the alveolar capillary barrier in the *ex vivo* perfused lung to injury by *t*-bu-OOH. This effect was measured by calculating the pulmonary capillary filtration coefficient (K_{fc}), a measure of the relative rate of fluid conductance across the microvascular bed.[14] The biochemical interaction between *t*-bu-OOH administration and halothane was detected by measurement of metabolites of the eicosanoid mediators TxA_2 and prostacyclin in effluent perfusate.

Materials and Methods

Preparation of the Isolated Perfused Rabbit Lung

Appropriate approval was obtained from institutional animal use committees for the experimental procedures. Male New Zealand white rabbits weighing 2–3 kg (Langshaw Rabbitry, Grand Rapids, MI) were maintained on Carnation Family Rabbit Chow (St. Louis, MO) and allowed water *ad libitum*. On the day of the experiment, a marginal ear vein was cannulated and 1,000 U/kg heparin was administered. Anesthesia was induced with 10 mg/kg ketamine and 2–3 mg/kg xylazine, administered intravenously.

A small left thoracotomy was made and the rabbit was exsanguinated by withdrawal of blood from the left and right ventricles, after which the chest was opened widely by means of a right thoracotomy and a midline incision with removal of the sternum. A tracheotomy was performed, and a stainless steel cannula was secured in the trachea with umbilical tape. Stainless steel cannulas were secured in the pulmonary artery, through a right ventriculostomy, and in the left atrium. The umbilical tape securing the pulmonary artery also included the aorta, thus eliminating flow through the bronchial circulation.

The lungs were ventilated with a mixture of 5% CO_2 in air (carrier gas), at a frequency of 20 breaths/min and a peak inspiratory pressure of 10–12 mmHg, with 1–2 mmHg positive end-expiratory pressure. After initiation of ventilation, the lungs were perfused with approximately 200 ml Ca^{2+}-free Krebs-Henseleit buffer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM glucose, and 4 mM ethylene glycol bis(β-aminoethylether) N,N,N′,N′-tetraacetic acid [EGTA]; *p*H adjusted to 7.4) at 35 ml/min with a roller pump (Sarns, Ann Arbor, MI) to clear the lungs of blood, followed by continuous perfusion with the same buffer in a recirculating fashion, also at 35 ml/min. This procedure reduced the perfusate hemoglobin concentration to levels undetectable using a hemoximeter (Model OSM3, Radiometer, Copenhagen, Denmark). The relatively low initial flow rate of 35 ml/min was chosen for the current experiments because this rate was used successfully in other experiments employing the same model.[8-10,12] In earlier experiments using this methodology and protocol, the lungs were perfused with buffer containing 2.5 mM Ca^{2+} and no EGTA.§ Attenuation of the pulmonary arterial pressor response to infusion of *t*-bu-OOH occurred in lungs ventilated with halothane relative to lungs ventilated with carrier gas. K_{fc} did not increase in lungs in the former group compared with lungs in the latter group. We speculated that, in these previous experiments, the attenuation of oxidant-induced pressor response by halothane protected the lungs against edema formation. The Ca^{2+}-free perfusate used in the present experiments completely eliminated the oxidant-mediated pressor response, thus removing this confounding hemodynamic variable. The perfusate was recirculated through a pulmonary venous reservoir suspended from a strain gauge (Grass model FT-10,

§ Shayevitz JR, Wade DD: Halothane and thiopental attenuate oxidant-induced pulmonary hypertension and edema formation in the isolated perfused rabbit lung (abstract). ANESTHESIOLOGY 69:A855, 1988.

Quincy, MA). The perfusate in the reservoir was bubbled gently with the same gas mixture used to ventilate the lungs, and the reservoir itself was covered with a snugly-fitting lid. The following values were recorded on a Gould 2600S polygraph (Cleveland, OH): pulmonary artery pressure (P_{pa}), pulmonary venous pressure (P_v), proximal airway pressure (P_{aw}), and pulmonary venous reservoir weight (W_L), which is a measure of lung water uptake. Perfusate temperature was maintained between 36° and 38° C by means of a heated water bath (fig. 1). P_v was initially maintained at −5 mmHg by adjusting the height of the pulmonary venous reservoir.

Experimental Protocol

Twenty-seven animals were used in these studies. At the end of a 20–30 min equilibration period when P_{pa} had stabilized, the perfusate flow rate was increased to 105 ml/min and P_v raised to +5 mmHg.[15] This flow rate (35 ml/kg/min) was chosen because (given the constraints of maximum pump rotor head speed and tubing size) tripling the baseline flow rate ensured an adequate and consistent P_{pa} difference. Matalon and Cesar (whose model ours imitates to some degree) used a flow rate approximately 20% higher during measurements of K_{fc}, which was, however, only twice their baseline flow.[16] At P_{aw} at which the lungs were ventilated, the mean P_{aw} was 3–4 mmHg (as noted on the chart recorder). Thus mean P_v was greater than mean P_{aw}, creating a zone 3 configuration, in which flow is independent of alveolar pressure. This P_v also produced, at least for the baseline recordings, a constant rate of fluid loss from the pulmonary venous reservoir. The W_L was recorded for 3 min, after which the flow was reduced to 35 ml/min until P_{pa} stabilized (usually 30 s). Subsequently, P_v was readjusted to −5 mmHg. A sample of perfusate was then withdrawn from the venous effluent and an infusion of *t*-bu-OOH was begun directly into the pulmonary artery at a final perfusate concentration of 200 μM for 1 min. Another sample of venous effluent was obtained 30 s after the *t*-bu-OOH infusion was stopped. We obtained perfusate samples at this time because earlier experiments demonstrated peak recovery of mediators from the pulmonary venous effluent approximately 30 s after cessation of the oxidant infusion.[12] These maneuvers were repeated three more times over the course of the experiment, with approximately 15 min elapsing between *t*-bu-OOH infusions. Previous experiments had shown that P_{pa} stabilized 5–7 min after the end of each *t*-bu-OOH infusion.[4,10,12] In no instance was P_{pa} changed by *t*-bu-OOH in Ca^{2+}-free perfusate. Just before the second increase in flow and P_v, simultaneous perfusate samples from the arterial inflow and the venous effluent were obtained from each experimental preparation for determination of *p*H, partial pressure of oxygen, and carbon dioxide tension with a Radiometer model ABL2 blood gas laboratory (Copenhagen, Denmark).

Lungs were treated in three ways. In group A (n = 9), lungs were ventilated with 2.5% halothane in carrier gas throughout the experiment, by means of a calibrated vaporizer (Fluotec Mark II, Cyprane, Keighley, England) filled with commercially available liquid halothane; in group B (n = 9), lungs were ventilated with carrier gas alone; and in group C (n = 9), lungs were ventilated with carrier gas alone and received infusions of vehicle (normal saline) rather than *t*-bu-OOH. The latter group formed the time controls for this series of experiments. Inspired halothane concentrations were confirmed with an infrared absorption gas analyzer (Traverse Medical Monitors, Traverse City, MI). All groups were treated concurrently but in sequence.

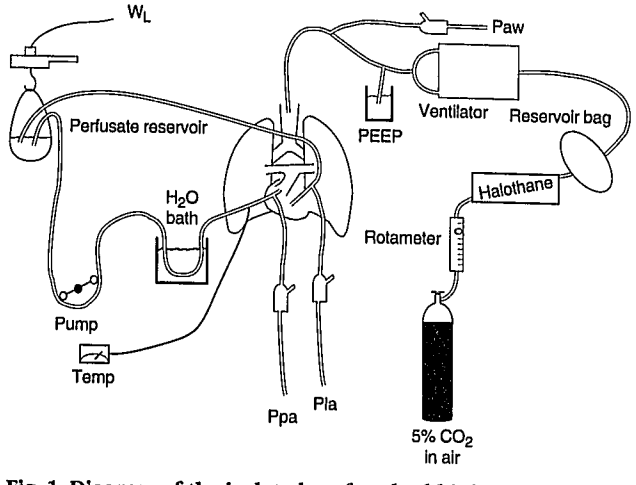

Fig. 1. Diagram of the isolated perfused rabbit lung model used in these experiments. P_{aw} = proximal airway pressure; P_{pa} = pulmonary artery pressure; P_{la} = pulmonary venous pressure; W_L = reservoir weight; PEEP = positive end-expiratory pressure. Perfusate circulates through the pulmonary vasculature by means of a roller pump (pump). Perfusate temperature (temp) is monitored at the pulmonary artery, and maintained at 36–38° C by means of a heated water bath (H_2O bath). A pulmonary venous reservoir (perfusate reservoir) suspended from a strain gauge acts as a monitor of lung water uptake. An in-line halothane vaporizer (halothane) is used to administer volatile anesthetic when appropriate.

At the end of the perfusion, the lungs were dissected from the thoracic cavity *en bloc,* and the trachea was excised 2–3 mm above the carina. After connective tissue and fat were removed, the lungs were weighed wet, dried to a constant weight (36–72 h) at 60–80° C, and reweighed. After each infusion of *t*-bu-OOH, K_{fc} was calculated using the method described by Matalon and Nickerson,[15] with the following formula:

$$K_{fc}(\text{mL} \cdot \text{min} \cdot \text{mmHg} \cdot 100 \text{ g dry tissue}) = \left(\frac{\frac{\Delta W_L \cdot 1.3}{\Delta P_{pa}}}{\text{Dry lung weight}} \right) \cdot 100,$$

where ΔW_L is the change in reservoir weight over the final minute of the 3-min period of elevated P_v; ΔP_{pa} is the difference in P_{pa} when the flow is 105 ml/min and then subsequently lowered to 35 ml/min; and 1.3 is the assumed ratio of pulmonary arterial to capillary pressure. The units of K_{fc} indicate that this coefficient expresses a rate of lung water uptake relative to an approximation of intracapillary hydrostatic pressure present at the time of measurement.

Mediator Assays

Concentrations of thromboxane B_2 (TxB_2), the stable metabolite of TxA_2, and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable hydrolysis product of prostacyclin, were determined in the samples of venous effluent by means of radioimmunoassays performed by the Michigan Diabetes Research and Training Center (University of Michigan, Ann Arbor, MI). The assay for TxB_2[17] employs a tritiated tracer (DuPont-New England Nuclear, Wilmington, DE), a rabbit-derived anti-TxB_2 antibody (Upjohn Company, Kalamazoo, MI), and dextran-coated charcoal for separation. The limit of sensitivity for the assay is 5 pg/ml. Interassay and intraassay variabilities are 8.0% and 2.8%, respectively. The crossreactivity of the anti-TxB_2 antibody is 1.5% for PGD_2 and less than 0.6% for all other relevant cyclooxygenase-derived arachidonic acid metabolites. The assay for 6-keto-$PGF_{1\alpha}$ (code RPA.515, Amersham Corporation, Arlington Heights, IL) employs a magnetic double-antibody technique, in which 6-keto-$PGF_{1\alpha}$-[^{125}I]-iodotyrosine is used as the tracer. A rabbit-derived anti-6-keto-$PGF_{1\alpha}$ antibody is the first antibody; and a donkey anti-rabbit serum second antibody, coated onto magnetizable polymer particles, is used for separation of free and bound ligand. The sensitivity limit of this assay is 15 pg/ml, and interassay and intraassay variabilities are 18.7% and 3.3%, respectively. Crossreactivity of the first antibody is 1% or less for prostaglandin $F_{2\alpha}$, prostaglandin E_2, prostaglandin $F_{1\alpha}$, prostaglandin E_1, and TxB_2. The rates of production of TxB_2 and 6-keto-$PGF_{1\alpha}$ were calculated as follows:

$$\text{Rate of production } (\text{pg} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \text{ dry tissue}) = \frac{(\text{mediator}_{post} - \text{mediator}_{pre}) \cdot 35}{\text{Dry lung weight}},$$

where mediator_{post} and mediator_{pre} represent the mediator concentration (pg/ml) after and before *t*-bu-OOH infusion, and 35 is the perfusate flow rate (ml/min) at the time the samples were obtained.

Statistical Analysis

All data are expressed as mean ± SE. Analysis of variance was used to compare groups. Scheffé's or Dunnet's test was used for *post hoc* comparisons where appropriate. When $\alpha \geq 95\%$ ($P \leq 0.05$), differences between groups were considered significant.

Results

No P_{pa} elevation was seen during perfusion with Ca^{2+}-free buffer, as demonstrated by a sample of a trace from a representative experiment, in which the lungs were injured with *t*-bu-OOH (fig. 2b). This trace is contrasted with one obtained from a representative experiment performed with Krebs-Henseleit buffer solution containing 2.5 mM Ca^{2+}, in which a marked transient elevation in P_{pa} is evident accompanying infusion of oxidant (fig. 2a). Baseline P_{pa} was similar in all groups of lungs, as was P_{pa} just before each of the four infusions of oxidant. Table 1 lists the values for P_{pa} during zone 3 conditions after each infusion of *t*-bu-OOH, when P_v and perfusate flow rate were elevated for determination of K_{fc}. All values were similar, and the presence of halothane had no effect.

Figure 3 is a plot of the mean percent change in K_{fc} over the *t*-bu-OOH infusion number for the treatment groups (injury plus halothane, injury plus carrier gas, and no injury plus carrier gas). Table 2 gives the absolute values for the mean K_{fc} obtained for all four infusions of *t*-bu-OOH in the three treatment groups. K_{fc} increased in a linear fashion over the course of the experiment in group A. In groups B and C, K_{fc} increased as well, but not to the degree seen in group A lungs. A statistically significant enhancement of K_{fc} is seen in group A lungs compared with group B and C lungs.

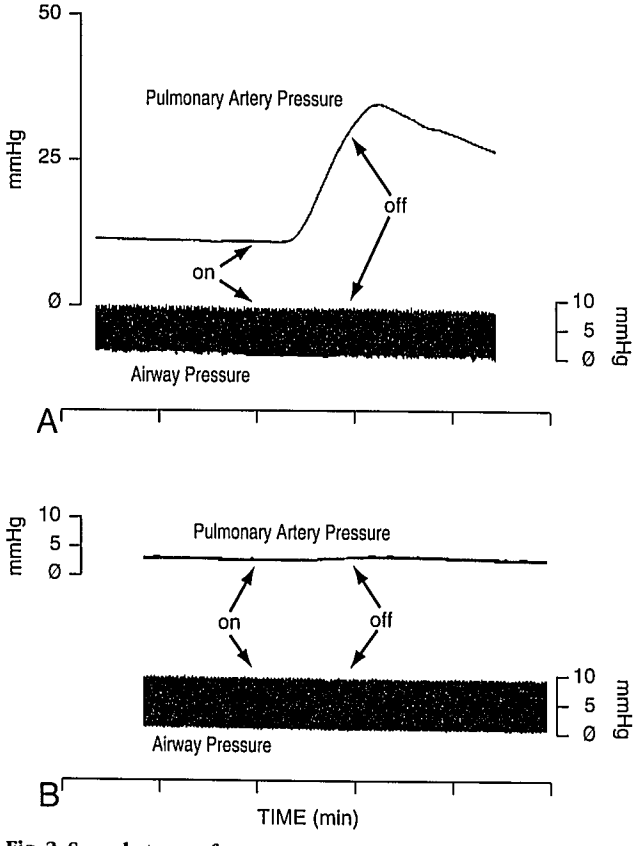

Fig. 2. Sample traces from representative experiments in which lungs were perfused with 2.5 mM Ca^{2+} (*A*) or Ca^{2+}-free buffer (*B*). On, off = onset, offset of *t*-bu-OOH infusion, respectively. At no time was there a significant change in P_{pa} in the lungs perfused with Ca^{2+}-free Krebs-Henseleit buffer solution.

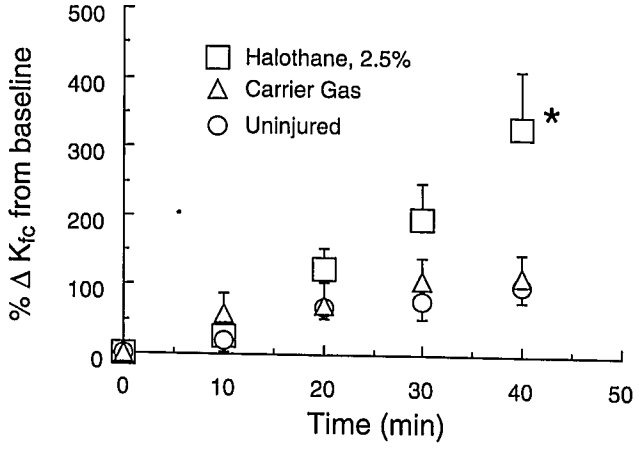

Fig. 3. Graph of percent change in K_{fc} from baseline *versus t*-bu-OOH infusion number, expressed as time. The difference between the two groups of lungs ventilated with carrier gas was not statistically significant. *Different from the injured and uninjured groups of lungs ventilated with carrier gas ($P < 0.03$). Vertical bars represent standard error of the mean.

Although the ratio of wet to dry weight was greater in group A (14.3 ± 0.7) than in group C (12.1 ± 1.1, $P < 0.05$) lungs, no difference was apparent between group B (13.2 ± 0.2) and group C.

In all lungs, little thromboxane was found in the pulmonary venous effluent in the basal state before injury (6.5 ± 2 pg/ml). 6-Keto-$PGF_{1\alpha}$ was present in the pulmonary venous effluent at concentrations more than 30 times those of TxB_2 in the basal state before injury (227 ± 19 pg/ml). As can be seen in figure 4, the ratio of the perfusate concentration of TxB_2 to that of 6-keto-$PGF_{1\alpha}$ increased after each infusion of *t*-bu-OOH in the injured lungs. No difference in TxB_2/6-keto-$PGF_{1\alpha}$ between group A and B lungs was detected, although the ratio was greater in groups A and B than in group C lungs ($P < 0.001$) after each oxidant infusion. The mean rate of production of 6-keto-$PGF_{1\alpha}$ was greater in group A than in group C lungs ($P < 0.011$ for each comparison); however, no difference in 6-keto-$PGF_{1\alpha}$ production was detectable between the lungs in groups A and B or B and C (fig. 5a). The mean rate of production of TxB_2 (fig. 5b) was greater in group A lungs compared with group B lungs ($P < 0.02$) after the last

Table 1. Pulmonary Artery Pressure (P_{pa}) during the High-flow and Pulmonary Venous Pressure State (Zone 3) Used to Determine Pulmonary Vascular Filtration Coefficient

Treatment	Baseline	Infusion 1	Infusion 2	Infusion 3	Infusion 4
2.5% halothane	7.6 ± 0.2	7.3 ± 0.3	7.6 ± 0.3	7.6 ± 0.2	8.5 ± 0.2
Carrier gas	7.7 ± 0.5	7.8 ± 0.5	8.0 ± 0.6	8.0 ± 0.5	7.8 ± 0.6
No injury	8.9 ± 1.0	8.2 ± 1.0	8.0 ± 1.0	8.9 ± 1.0	9.8 ± 1.0

Values are mean ± SE for P_{pa} (mmHg). Data were obtained during each of the five zone 3 periods during which flow and pulmonary venous pressure were increased. Baseline values are those obtained during the equilibration period; Infusion 1 values, after the first oxidant infusion; Infusion 2 values, after the second oxidant infusion; and so on. By analysis of variance, no significant differences among groups occurred over the course of the protocol. Neither halothane nor successive infusions of *tert*-butyl-hydroperoxide have an effect on pulmonary vascular resistance or, therefore, vascular cross-sectional area, in this model.

Table 2. Pulmonary Vascular Filtration Coefficient (K_{fc}) for the Three Treatment Groups

Treatment	Preinjury	Infusion 1	Infusion 2	Infusion 3	Infusion 4
2.5% halothane	38.9 ± 7.1	43.6 ± 4.4	72.0 ± 12.9	98.5 ± 18.8	141.4 ± 27.2
Carrier gas	43.8 ± 12.0	58.6 ± 13.4	58.1 ± 10.2	67.5 ± 7.0	68.0 ± 5.6
No injury	32.6 ± 7.9	37.1 ± 8.9	44.3 ± 9.1	52.9 ± 11.4	63.9 ± 16.0

Values are mean ± SE for K_{fc}, expressed in $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100$ g dry tissue for the three treatment groups in relation to the *tert*-butyl-hydroperoxide infusion number. Preinjury represents the baseline value for K_{fc}. Note the consistently greater value for K_{fc} in the injured lungs ventilated with halothane compared with the other two groups of lungs. Although the K_{fc} in the halothane group is greater ($P < 0.05$) than the preinjury value after the final three oxidant infusions, the difference between the halothane group and the injured carrier gas group does not reach statistical significance until after the final oxidant infusion ($P < 0.03$). Also, the increase in K_{fc} in the carrier gas and no injury groups after infusion of oxidant or vehicle, respectively, was not significantly greater than baseline.

two infusions of *t*-bu-OOH. Lung production of TxB_2 (but not of 6-keto-$PGF_{1\alpha}$) increased with each infusion of oxidant in both group A ($P = 0.033$) and group B ($P = 0.002$).

Discussion

We performed these experiments to determine whether, in oxidant-injured lungs, halothane influences an indicator of lung fluid conductance, K_{fc} (a physiologic effect), and production of arachidonate-derived mediators by the lung (a biochemical effect), when the lungs are challenged repeatedly by an oxidant, *t*-bu-OOH. We hypothesize no cause-effect relationship between these two processes: the relationship between arachidonate-derived mediator production and lung injury has not been demonstrated consistently from species to species. We propose, however, that events common to the maintenance of the integrity of the endothelial barrier and to turnover of arachidonic acid may be affected by halothane and suggest possible targets for this halothane effect. Our findings demonstrate that injured, isolated lungs perfused with blood- and Ca^{2+}-free buffer exhibit a greater increase in K_{fc} while being ventilated with 2.5% halothane in carrier gas than with carrier gas alone. The higher K_{fc} in injured lungs ventilated with halothane is accompanied by a more rapid turnover of arachidonic acid in the presence of halothane plus injury than in injury alone upon infusion of *t*-bu-OOH.

The results of earlier studies using *t*-bu-OOH-injured perfused rabbit lungs suggest that the transient pulmonary arterial hypertension induced by the oxidant is mediated by TxA_2. When the lungs are perfused with inhibitors of phospholipase A_2,[18] cyclooxygenase,[12] or thromboxane synthetase,[10] the pressor response to *t*-bu-OOH is attenuated, suggesting that inhibition of TxA_2 release prevents the pressor response to oxidant. Similarly, when the thromboxane analog U46619 is infused, a transient elevation in P_{pa} is obtained.[19] The pressor response appears to depend on extracellular calcium primarily, because perfusion of the lungs with nominally calcium-free perfusate also results in attenuation of the pressor response.[10] On the other hand,

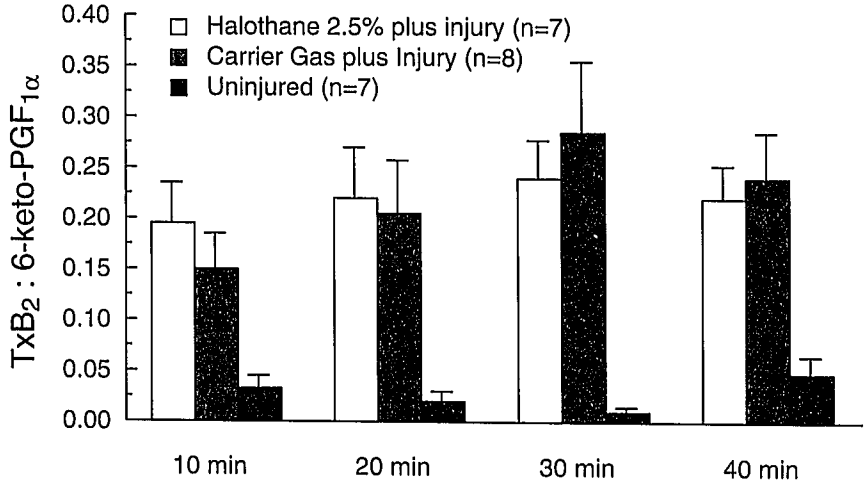

Fig. 4. Plot of the ratio of TxB_2 to 6-keto-$PGF_{1\alpha}$ for each infusion of *t*-bu-OOH, expressed as time. As previously reported,[4] infusion of *t*-bu-OOH into the pulmonary artery is associated with marked enhancement of the recovery of TxB_2 from the effluent perfusate relative to 6-keto-$PGF_{1\alpha}$. Vertical lines represent standard error of the mean.

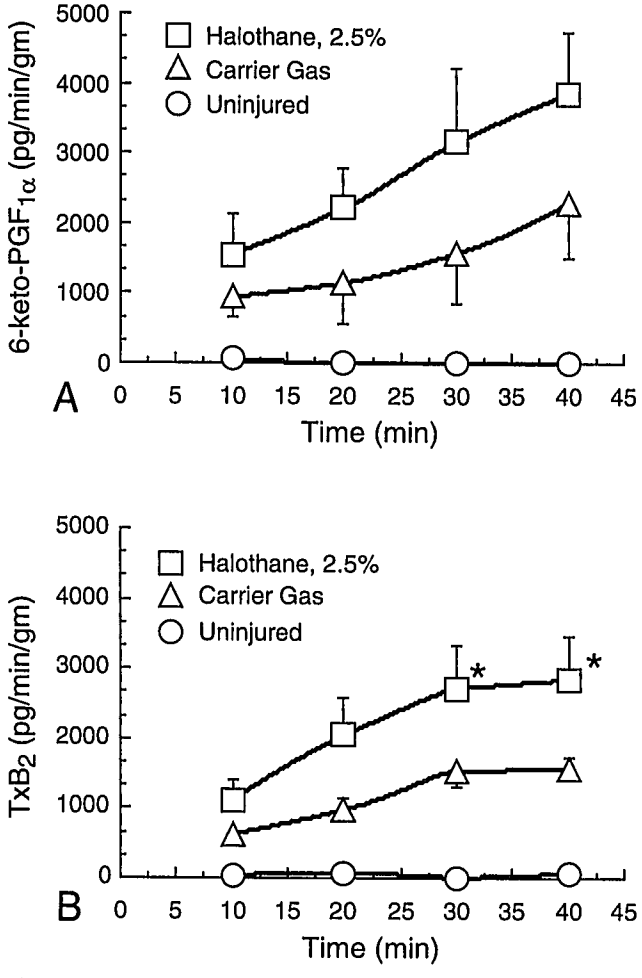

Fig. 5. Plots of the peak rates of production (mean $pg \cdot min^{-1} \cdot g^{-1}$ dry lung tissue ± SE) of 6-keto-$PGF_{1\alpha}$ (*A*) and TxB_2 (*B*) during infusion of *t*-bu-OOH into the pulmonary artery of the perfused rabbit lung. Vertical bars represent standard error of the mean. Overall, production of TxB_2 was statistically greater in the injured lungs ventilated with halothane than in either of the other groups of lungs ($P < 0.02$). A significant difference was seen by the final infusion of *t*-bu-OOH between injured lungs ventilated with halothane and those ventilated with carrier gas ($P < 0.02$). Although production of 6-keto-$PGF_{1\alpha}$ was consistently greater in the injured lungs ventilated with halothane, this difference never reached statistical significance, because of the variability within groups.

the turnover of arachidonic acid in response to *t*-bu-OOH may depend on mobilization of intracellular calcium, as seen in cultured bovine pulmonary vascular endothelium[20] and in perfused lungs,[10] in which arachidonate mediator production occurred during oxidant treatment despite the lack of extracellular calcium. Thus, in the absence of extracellular calcium, the stimulus (infusion of *t*-bu-OOH) is uncoupled from the response (transient pulmonary hypertension). Although perfusion of the lungs with Ca^{2+}-free buffer may alter other physiologic responses to halothane, processes dependent on mobilization of intracellular Ca^{2+} may remain unaffected.

The present experiments and others provide evidence for an interaction between halothane and *t*-bu-OOH-stimulated arachidonate-derived mediator production. Volatile anesthetics enhance the pressor response to infused *t*-bu-OOH concomitant with augmented recovery of TxB_2 from the effluent perfusate.[4] Similarly, the results of the present studies indicate further that higher rates of production of TxB_2 (a stable metabolite of TxA_2, which has vasoconstrictor and thrombogenic properties) and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin, which has vasodilator and antithrombogenic properties) occur during infusion of *t*-bu-OOH in halothane-treated lungs relative to the lungs in the other treatment groups. Simultaneously, however, the ratio of the absolute TxB_2 to 6-keto-$PGF_{1\alpha}$ concentrations in the pulmonary venous effluent remains similar in the two groups of injured lungs. To achieve higher rates of mediator production while concentration ratios remain unchanged, injured lung tissue must release greater amounts of free arachidonate when ventilated with halothane in carrier gas than when ventilated with carrier gas alone.

The turnover of arachidonic acid requires phospholipase A_2, which is dependent for activity on the free intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration. *T*-bu-OOH treatment of cultured bovine pulmonary artery endothelial cells stimulates phospholipase A_2 activity and TxB_2 production, even if the extracellular buffer is nominally Ca^{2+}-free.[20] Oxidant-stimulated arachidonic acid turnover and metabolism and intracellular calcium mobilization are well documented phenomena. Exogenously administered hydrogen peroxide has been shown to stimulate release of endothelial cell-derived arachidonic acid.[21] Similarly, Taylor *et al.* have demonstrated that low concentrations of hydroperoxides (such as *t*-bu-OOH) stimulate release of prostacyclin from fibroblasts.[22] Hydrogen peroxide increases $[Ca^{2+}]_i$ primarily by stimulating release of Ca^{2+} from internal stores,[20,23] most likely *via* phospholipase C-mediated phosphatidylinositol hydrolysis, and subsequent release of inositol triphosphate. Similar mechanisms may apply to the hydroperoxides, such as *t*-bu-OOH, although phospholipase C may not be involved in the *t*-bu-OOH effect.[19,24]

In single rat pulmonary artery endothelial cells, halothane and isoflurane enhanced the release of sequestered intracellular Ca^{2+} associated with peroxide injury.‖ Thus, if phospholipase A_2 activation is Ca^{2+}-dependent, then it is conceivable that more of this phospholipase is activated in the presence of halothane than in the presence of carrier gas alone. These considerations may account for the augmented production of TxB_2 in lungs injured with *t*-bu-OOH after equilibration with halothane. We were not as successful in detecting significant changes in production of 6-keto-$PGF_{1\alpha}$, primarily because of the larger intragroup variability in 6-keto-$PGF_{1\alpha}$ determinations.

The present study also suggests an interaction between halothane and oxidant on decreasing the integrity of the alveolar-capillary barrier in the lung. In recent *in vitro* experiments, we reported increased sensitivity of halothane-exposed endothelial cell monolayers to injury mediated by stimulated neutrophils.[1] This enhanced injury was not accompanied by a change in the rate of production of hydrogen peroxide by the neutrophils. Similar but less consistent results were observed in monolayers injured with reagent hydrogen peroxide, especially at the highest concentration of halothane studied (2.5%). The present data also demonstrate that, in perfused lungs, an index of the fluid conductance of the pulmonary vascular bed, K_{fc},[14] is increased in injured lungs ventilated with halothane but not significantly in injured lungs ventilated with carrier gas alone, relative to control, uninjured lungs. Although the increase in K_{fc} in halothane-treated lungs did not achieve statistical significance when compared with injured lungs ventilated with carrier gas alone until after the fourth and final infusion of oxidant, K_{fc} increases with each *t*-bu-OOH infusion in the former group ($P = 0.0004$) but not in the latter. We hypothesize that the pulmonary vascular endothelium is a likely target of halothane in increasing this injury index, because (1) in *in vitro* studies, endothelial monolayer injury enhancement occurred regardless whether neutrophils were present, in the presence of halothane; (2) the *ex vivo* lungs were perfused with clear buffer, containing few blood cells (hemoglobin concentration was too low to be detected by hemoximeter), thus decreasing the possibility that blood-borne inflammatory cells contributed to this effect; and (3) microvascular endothelial disruption must occur (due to an increase in capillary "pore" size or number) for fluid movement to occur, such as evidenced by the change in K_{fc}. In using this isolated lung model, we make certain assumptions about alveolar-capillary membrane characteristics that affect K_{fc}: (1) the viscosity of the perfusate is assumed to remain constant, (2) the thickness of the capillary wall is assumed not to change after the initial period of equilibration, and (3) the surface area of the perfused capillary bed also is assumed to remain constant and similar among the three treatment groups because, as noted previously (table 1), no differences in perfusion pressure under baseline or zone 3 conditions occurred among the three groups.

We acknowledge that the response of the lung as a whole to oxidant may be altered in the presence of nominally Ca^{2+}-free perfusate. Indeed, endothelial cell monolayers bathed in 100 nM Ca^{2+} buffer exhibit alterations in shape, marked by rounding and loss of spreading, compared to cells bathed in 1.8 mM Ca^{2+} buffer.‖ Thus, in nominally Ca^{2+}-free perfusate, lungs may exhibit enhanced fluid transfer from the intravascular to the extravascular space because of diminished cell–cell contact and enlargement of pore size.[25] These considerations partially may explain the 10-fold greater K_{fc} seen in our experimental preparation, compared to that noted by Matalon and Cesar in rabbit lungs perfused with colloid-containing physiologic buffer.[15] Another difference between our experimental preparation and that described by Matalon and Cesar, which may partially contribute to the greater K_{fc} in our model, is the absence of colloid in the perfusate used in our experiments.[25] We attempted to perfuse lungs with colloid (bovine serum albumin)-containing buffer in developmental stages of the model, but were unable to obtain a stable system.

Although we detected differences in K_{fc} among the three groups of lungs, our data for lung wet/dry ratios did not show parallel changes consistently. The mean wet/dry ratio in the uninjured lungs was less than in either of the other two groups, but there was no difference between the two groups of injured lungs. The lack of agreement between these two methods of quantifying lung injury implies that the wet/dry ratio and K_{fc} are neither directly nor simply related. The wet/dry ratio represents a static determination of the accumulation of fluid throughout the lung, not only in the interstitium but also in the alveolar space. In principle, K_{fc}, as described by Taylor and Townsley, is "a

‖ Shayevitz JR, Varani J, Knight PR: Modulation of intracellular calcium fluxes in oxidant-injured endothelial cells by halothane and isoflurane (abstract). ANESTHESIOLOGY 73:A341, 1990.

permeability parameter describing the volume-flow characteristics of the capillary wall."[14] K_{fc} directly depends on the number of holes present in this wall, the size of the holes, and the surface area available for fluid exchange. Thus, a few large holes in lungs with a given capillary surface area may not have as great an effect on K_{fc} as many smaller leaks across the same size surface. Lung wet/dry weight ratio, however, may change the same amount under these different conditions, especially if the capillary surface area remains unchanged.[14] Thus, the discrepancy between the data for wet/dry weight ratio and K_{fc} may reflect a difference in the quality of injury between the two groups of injured lungs, if we assume no effect of halothane on the volume-flow characteristics of the microvascular bed.[14] Halothane, perhaps by increasing the sensitivity of a broader spectrum of microvascular endothelium to the injury incurred by *t*-bu-OOH (as indicated by our *in vitro* results[1]), may affect the distribution of oxidant-mediated injury. In lungs ventilated with carrier gas alone, injury may occur in limited, localized regions, thus creating a few larger holes. Injury in lungs ventilated with halothane may be more diffuse and severe than injury in carrier gas-ventilated lungs, thus creating a larger number of smaller holes. In addition, ventilation with halothane may act to increase the vascular capacitance of the isolated lung, which also may be reflected as a higher K_{fc}; but this is arguable for several reasons: (1) the baseline and zone 3 P_{pa} in all groups of lungs were similar; (2) the preinjury K_{fc} in the three groups of lungs was the same; (3) the reactivity of the pulmonary vasculature in response to numerous stimuli in lungs perfused with Ca^{2+}-free buffer is markedly attenuated, if not completely eliminated[10]; and (4) with the change in venous pressure and flow used to measure K_{fc}, the effect of increased vascular capacitance on lung perfusate uptake is short-lived, usually lasting not longer than the first 30 s after these parameters are changed.[15,25]

The augmented TxB_2 production and simultaneous injury enhancement in halothane-exposed lungs may be related to an effect of halothane on peroxide-mediated mobilization of intracellular Ca^{2+}. In several cell lines (both endothelial and epithelial) known to form tight junctions, exposure to oxidants has been shown to induce cell contraction and separation of tight junctions and an increase in electrical conductance across cell monolayers.[26-29] This effect is accompanied by disruption of the F-actin cytoskeleton,[26] mobilization of intracellular Ca^{2+},[27] and a decrease in intracellular ATP levels.[27-29] Cytoskeletal disruption with resultant loss of tight junctions (and increased permeability of monolayers) may be mediated by oxidant-stimulated intracellular Ca^{2+} mobilization.[26,27] Thus, by promoting intracellular Ca^{2+} mobilization in oxidant-injured endothelium,‖ halothane may contribute to the enhancement of permeability (K_{fc}) simultaneous with stimulation of arachidonic acid turnover in the perfused lungs described in the present experiments.

Ex vivo perfused rabbit lungs ventilated with halothane simultaneously exhibited evidence of greater fluid conductance across the pulmonary capillary bed and production of TxA_2 when challenged with organic oxidant than did lungs ventilated with carrier gas. Both of these effects may be mediated by halothane-related enhancement of intracellular endothelial Ca^{2+} mobilization stimulated by intrapulmonary infusion of oxidant. These effects of halothane in perfused lungs may broaden our understanding of volatile anesthetic interactions with acute inflammatory lung injury resulting from such processes as oxygen toxicity, adult respiratory distress syndrome, multiple organ failure syndrome, and acid aspiration.

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