Effect of smoke inhalation on viscoelastic properties and ventilation distribution in sheep

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Smoke inhalation is a multifaceted injury with both direct and indirect pulmonary injury. In the clinical scenario, absorption of heat in the oropharynx induces cellular necrosis and capillary leakiness (23). The majority of the thermal energy is dissipated here by the highly vascularized structures, and the injury caused to the lower airways and pulmonary parenchymal tissue is the result of irritant gases and particulate debris. Any material combusted in an oxygen-poor environment may result in products of incomplete combustion, namely carbon monoxide and, less commonly, cyanide (carbon and nitrogen, respectively). Hence upper airway damage, carbon monoxide poisoning, and potentially cyanide poisoning precede the development of the delayed acute lung injury, which results in the requirement for prolonged mechanical ventilation, a phenomenon seen in the majority of severe smoke inhalation injuries. One of the major disadvantages of using a smoke inhalation injury for ARDS is the difficult application and the accurate “dosing” of smoke delivered. Most studies used in a combined smoke inhalation with a burn injury or a bacterial sepsis to enhance the inflammatory response of the lung.

Furthermore, there are no studies describing the time course of changes in viscoelastic properties and ventilation distribution of the lungs after smoke inhalation only. The majority of patients who suffer from severe smoke inhalation almost invariably require mechanical ventilation at some point in their hospital stay; hence the understanding of changes of mechanical characteristics and gas distribution after smoke inhalation is essential to optimize respiratory management. Adequate oxygenation may be difficult to obtain, as a result of regional hypoventilation, ventilation-perfusion mismatch, alveolar collapse due to deficiencies in surfactant, and noncardiogenic pulmonary edema. It can be difficult to predict in a patient with multiple pathologies the etiology of changes in oxygenation. Furthermore, there is a deficiency in practical diagnostic tools to optimize respiratory support in smoke inhalation injury. Chest X-rays give limited one-dimensional information; bronchoscopy is invasive and can result in worsening hypoxemia and pneumothorax; and computerized tomography (CT) scanning of the chest is expensive and time consuming and transfer...
from the intensive care unit can lead to cardiorespiratory embarrassment in an already compromised patient.

The intention of this study was to investigate to measure the impact of smoke inhalation on viscoelastic properties and ventilation distribution of the lung within the first 2 h after smoke exposure, while examining the efficiency and reproducibility of a new smoke inhalation device.

METHODS

Smoke Inhalation Model

Animal preparation. The study has been approved by the local animal welfare and ethics committee (University of Queensland, Brisbane, Australia), and the experiments were performed according to the Guide and Care for the Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, 1985). Fifteen Merino ewes were housed in outdoor pens at our institution. They were fasted for 24 h, before induction of anesthesia with 150–250 mg propofol and intubation of the larynx with size 10 Portex (SIMS Portex, Keene, NH) cuffed endotracheal tube. Anesthesia was maintained with continuous propofol infusion (80–120 ml/h) and supplemented with intravenous buprenorphine 0.01 mg/kg. Under sterile conditions, a triple-lumen boulus was passed through the bronchoscope. Anesthesia was maintained with continuous propofol infusion (80–120 ml/h) and supplemental inhalation of oxygen. A central venous catheter was sited in the right internal jugular vein and connected to plastic tubing. The plastic tubing is then connected to the bellows device with a T piece and the expiratory port is manually occluded during expiration.

Ventilation before and after smoke inhalation. All animals (smoke and sham animals) were mechanically ventilated during the whole experiment with zero positive end-expiratory pressure with a constant tidal volume of 10 ml/kg, an inspiratory oxygen fraction of 1.0, and a respiratory rate of 20/min using a Hamilton Galileo ventilator (Rheinz, Switzerland).

Electrical impedance tomography. Electrical impedance tomography (EIT) is a relatively new technique generating cross-sectional images of the studied subject on the basis of the measurement of surface electrical potentials resulting from an excitation with known small electrical currents (5 mA and 50 kHz). Both the voltage measurements and current injections take place between pairs of electrodes of a 16-electrode array attached on the chest circumference. The electrical currents were applied to the skin of the sheep, at regular intervals to obtain optimal signal. EIT scans are generated from the collected potential differences and the known excitation currents by using weighted backprojection in a 32 × 32 pixel matrix (6). Each pixel of the scan shows the instantaneous
local impedance. A Göttingen high-performance EIT (Sensor Medics, Viasys, The Netherlands) has been used with a frame rate of 12.5 frames/s.

Data Analysis

MBW. Software associated with the ultrasonic flowmeter was used to calculate the FRC, analysis of ventilation distribution homogeneity, and measurement of dynamic compliance. Flow and volume were corrected btps according to the humidity and temperature inside the ventilator circuit. The ultrasonic flowmeter measured the molar mass of the breathing gas. The molar mass change was calculated by subtraction of tidal breathing baseline and thus SF6 fraction was obtained. The SF6 flow was calculated as the product of airflow and SF6 concentration for each sampled pair of measured values (20). First the lungs were equilibrated with ~6% SF6. Subsequently during expiration the SF6 supply was switched off. Expiratory SF6 flow was integrated over time, giving the expired SF6 volume. FRC of the washout was obtained by dividing the measured exhaled SF6 volume with the initial SF6 volume within the flowmeter and the connectors was subtracted from the total of the exhaled SF6 volume. Ventilation distribution homogeneity was analyzed using lung clearance index (LCI) and mean dilution number (MDN) analysis of the SF6 washout curve. LCI is the number of volume turnovers required to reduce end-tidal SF6 concentration to 1/40 of the tracer concentration at the start of the washout (11). The MDN is the ratio between the first and the zeroth moments of the washout curve (11, 21). Any increase in value of both indexes (LCI and MDN) indicates an increased ventilation mal-distribution.

EIT. The reference state used for EIT was tidal ventilation using a tidal volume of 10 ml/kg in right lateral position. EIT was measured during 1 min periods. To quantify the magnitude of local ventilation distribution, two different methods for EIT data analysis (EIT profiles and EIT time course analysis) were used. First, EIT profiles of local ventilation distribution were generated. For this purpose EIT data were analyzed by calculating the mean relative impedance change of any pixel for the 1-min measurement period. On the basis of the 32 local ventilation distribution were generated. For this purpose EIT data and EIT time course analysis) were used. First, EIT profiles of local ventilation distribution were generated. For this purpose EIT data were analyzed by calculating the mean relative impedance change of any pixel for the 1-min measurement period. On the basis of the 32 x 32 pixel matrix the mean relative impedance change of 32 rows starting from the independent to the dependent lung was calculated and a profile of relative impedance change generated (18). The amplitude of relative impedance change for each lung was expressed as a percentage of the global relative impedance change. Second, the time course of relative impedance change during the EIT measurement was analyzed for the global, the left, and the right lung. An example of relative impedance change obtained in a sheep is displayed in Fig. 2A. Cardiac oscillations of the EIT signal were filtered with a low-pass filter at 0.5 Hz. Impedance changes of left and right lung were then plotted against global lung impedance change in a xy-graph (Fig. 2B). Ideally the plotted loop of the left and the right lung should cross the x-axis through zero with a 45° angle, indicating that both lungs are equally ventilated. An angle <45° indicates that one lung is less well ventilated and an angle >45° indicates that one lung is better ventilated than the other.

For data acquisition and reconstruction of functional EIT images, software provided with the equipment was used (4). Functional EIT data were further analyzed offline by using Math_Lab 7.1 (The MathWorks).

Lung mechanics. Resistance and dynamic lung compliance during tidal ventilation were assessed with a multiple-regression technique. Pressure and flow data were analyzed on a breath cycle-per-cycle basis. After identification of a cycle, the flow signal was corrected for any offset, by using the assumption that inspired and expired volumes were identical. The pressure was then corrected for the pressure drop along the endotracheal tube (10). At least 10 breaths were analyzed for one compliance or resistance value. The stress index (SI) obtained from the pressure-time waveform was calculated before and 120 min after smoke inhalation injury (9, 17). A nonlinear fit is applied to the pressure-time waveform using the formula P(t) = a·t^b + c, where a and c are constant coefficients and b = SI. SI measured during tidal breathing indicates whether the lung compliance increases (SI < 1.0) or decreases (SI > 1.0) during a tidal breath and discriminates between tidal recruitment and tidal hyperinflation. Smoke inhalation causes surfactant depletion (14), and therefore we speculated that, as tidal compliance decreases, the value of SI increases.

Blood-gas analysis. Arterial blood samples were drawn before smoke inhalation and 5 min and 120 min after smoke. Carboxyhemoglobin (COHb) percent levels and arterial oxygen tension (PaO2) were measured in a blood-gas analyzer machine (ABL System 625, Brisbane, Australia).

Statistics

Results are given as means ± SE for parametric data. A Student’s paired t-test was used to compare values before and after smoke inhalation injury. Repeated-measures analysis of variance with a post hoc Bonferroni correction for multiple comparisons was used to determine the effects of group-time interaction for each parameter measured repeatedly. Statistical significance was accepted at a P < 0.05 for all comparisons.
RESULTS

Reproducibility of the Smoke Inhalation Model

Ten sheep with an average body weight of 34.1 kg (range 27–43 kg) were exposed to smoke inhalation injury. Measured COHb before smoke inhalation was 3.62 ± 0.27% and 5 min after smoke was 61.1 ± 1.9%, range 50–69.4% (P < 0.001). PaO₂ before smoke inhalation was 448 ± 11 Torr and 5 min after smoke exposure was 364 ± 26 Torr (P not significant (NS)). Two hours after smoke inhalation, the PaO₂ dropped to 200 ± 40 Torr and the COHb was 8.0 ± 0.4% (P < 0.001).

Eight of ten sheep had a PaO₂-to-inspired oxygen fraction (FIO₂) ratio of <200 after smoke exposure. The five sheep of the sham group had an average body weight of 32.6 kg (range 29–36 kg). In the sham group the PaO₂, remained unchanged (461 ± 29 Torr before and 434 ± 33 Torr after sham smoke, P = NS) and the COHb as well remained unchanged (4.8 ± 0.9% before and 5.2 ± 0.9% 5 min after sham smoke). Physiological data on recorded blood pressure and heart rate are plotted in Fig. 3. None of the animals died because of the smoke inhalation injury.

Lung Mechanics

Peak inspiratory pressures increased in from 12.1 ± 1.0 to 16.8 ± 1.4 cmH₂O (P < 0.05), whereas in the sham group the pressures remained unchanged (11.8 ± 0.8 and 11.6 ± 0.9 cmH₂O, respectively). Measured dynamic compliance decreased from 56.6 ± 5.5 to 32.8 ± 3.2 ml/cmH₂O (P < 0.05), and respiratory resistance increased from 3.4 ± 0.3 to 4.5 ± 0.5 cmH₂O·l⁻¹·s (P < 0.05) (Fig. 4). In the sham group, compliance was the same before and after sham smoke procedure (58.6 ± 8.5 to 57.8 ± 7.2 ml/cmH₂O), and the airway resistance as well remained unchanged (3.3 ± 0.5 to 3.5 ± 0.5 cmH₂O·l⁻¹·s). The SI increased from 0.994 ± 0.009 to 1.081 ± 0.011 (P < 0.01). In the control group, the SI remained unchanged 0.986 ± 0.012 to 0.991 ± 0.021.

Ventilation Distribution

FRC decreased from 1,773 ± 226 ml before smoke to 1,006 ± 129 ml 2 h after smoke inhalation (P < 0.05). FRC in the sham sheep remained unchanged. Ventilation inhomogeneity increased with a LCI of 10.4 ± 0.4 and MDN of 2.8 ± 0.1 to a LCI of 14.2 ± 0.9 and MDN of 3.9 ± 0.3 2 h after smoke exposure (P < 0.05) (Fig. 5). LCI and MDN in the sham group remained unchanged. Figure 6 shows the profiles of local relative impedance change obtained before and after smoke inhalation. Before smoke inhalation the dependent (right) lung was preferentially ventilated, whereas after smoke exposure more ventilation was directed to the independent (left) lung. Analysis of the amplitude of relative impedance change showed that ventilation was directed more into the independent lung after smoke inhalation. The right lung was ventilated with 53.6 ± 6.0% and the left lung 46.4 ± 6.4% (P < 0.05) before smoke, and 2 h after smoke inhalation the right lung was ventilated with 32.1 ± 4% of the tidal volume and the left lung with 67.9 ± 8.2% (P < 0.001). The right lung of the sham group was ventilated with 53.3 ± 4% and the left lung with 46.7 ± 6.1% at the beginning of the experiment, and
2 h after sham smoke inhalation the right lung was ventilated with 52.1 ± 4% and the left lung with 47.9 ± 6.1% (P = NS). Before smoke inhalation, the phase angle for the right lung was 47.4 ± 1.8° and the left lung 41.6 ± 1.6° (P < 0.05). Two hours after smoke inhalation, the measured angle for the right lung was 32.2 ± 3.0° and for the left lung was 52.5 ± 1.3° (P < 0.001). In the sham group, the phase angles did not change (right lung with 46.1 ± 1.6°, left lung 41.6 ± 1.6° and after sham smoke 47.1 ± 1.9°, left lung 43.5 ± 1.2°). In all four sheep with continuous blood-gas analysis the PaO2 steadily decreased after smoke inhalation (Fig. 7).

**DISCUSSION**

In the present study we investigated the physiological changes and impact of smoke inhalation injury on pulmonary mechanics and gas distribution using a new smoke inhalation device in sheep. The pulmonary dysfunction can be summarized with a decrease of viscoelastic properties, decrease in ventilated lung volume, and increase in ventilation inhomogeneity. Furthermore, the ventilation was shifted from the dependent lung into independent lung regions, which were less exposed and hence less damaged by smoke inhalation. As early as 2 h after smoke inhalation significant gas exchange impairment with a PaO2-to-FIO2 ratio of <300 was observed.

The histopathological changes and inflammatory response of smoke inhalation are well investigated and have recently been reviewed by Enkhbaatar and Traber (5). In summary, smoke inhalation causes pulmonary edema due to direct tissue injury, airway obstruction due to mucus plugging, and bronchoconstriction and increased pulmonary shunt fraction due to impairment of the hypoxic pulmonary vasoconstrictive response. In the past, most smoke inhalation studies used a combined smoke inhalation and burn injury or bacterial sepsis (1, 12).
Our study aimed to investigate the specific effect of smoke inhalation on the viscoelastic properties and ventilation distribution.

Reproducibility of the New Smoke Inhalation Device

The new smoke device has a closed system with minimal smoke escaping into the environment, guaranteeing optimal and more importantly reproducible smoke inhalation. The amount of cotton burnt can be exactly be defined with the new smoke device. Because the cotton is combusted within the device, no products of combustion are lost to the environment (as was seen in our previous device), which we believe has enabled us to create a highly reliable injury. All animals had very consistent high COHb levels and highly reproducible pulmonary dysfunction after smoke exposure. There are only few reports describing similar smoke inhalation in animal models. Traber’s group in the Shriner’s Hospital at Galveston, Texas, has recently reported their smoke inhalation model and found similar decrements in oxygenation, in combination with a 40% body surface area burn (1) or with Pseudomonas sepsis (12). In their study they used a modified bee smoker and delivered 48 breaths of smoke to the sheep. Significant impaired gas exchange occurred after 12–24 h. With the new smoke inhalation device no fuel is combusted and lost to the environment, which may partially explain the rapid onset of impaired gas exchange.

Ventilation Distribution After Smoke Injury

Animals were positioned on their right lateral side for the experiment directing most of the tidal ventilation into the dependent (right) lung (7). During smoke exposure more smoke was directed into the better ventilated (right) lung. We
have intentionally chosen a lateral position to provoke an inhomogeneous, predominately right-sided lung injury. In the past, ventilation distribution has been investigated with two fundamentally different techniques: inert-gas mixing techniques such as multiple-breath nitrogen washout, or imaging techniques such as CT. Each of these techniques is associated with specific models and experiments and thus is intrinsically limited. Previously CT scans in smoke-exposed animals have shown to be highly accurate to determine early in the course the severity of the smoke-induced lung injury (15). Histological examination postmortem revealed substantial parenchymal injury and damage to the larger airways. Most of the smoke inhalation injury occurred in the dependent lung regions, which has been confirmed in another study measuring the oxidative stress due to smoke inhalation (16). Both these studies are supported by the EIT measurements in our study. The dependent regions suffered more from smoke inhalation injury, and ventilation was shifted from the dependent to the nondependent lung as a result of it. EIT has not as high resolution as CT scans to quantify the degree of smoke inhalation injury, but it certainly can identify on a functional basis which regions of the lungs are mostly affected by the smoke inhalation injury.

EIT has recently emerged as a new imaging technique for bedside monitoring of ventilation distribution. EIT can identify and image spatial impedance changes associated with local air filling and emptying of the lungs during spontaneous and mechanical ventilation (6). EIT accurately measures changes in end-expiratory level and describes regional ventilation distribution by measurement of local impedance change. Frerichs et al. (8) showed that time-course analysis of local impedance change in selected lung areas is a good estimate for local tidal volume change. We chose to use EIT over CT scanning for the assessment of ventilation distribution because it can be instituted at the bedside, avoids repeated doses of radiation, and avoids the risks inherent in transferring an unstable subject to the CT scanner. Our data underline the validity of EIT to monitor changes in ventilation distribution (Fig. 8). The data of the MBW showed that ventilation distribution rapidly deteriorated, initially without major change in FRC. In the following 2 h after smoke inhalation, the ventilated lung volume gradually decreased, possibly because of pulmonary edema and mucus plugging of larger airways, bronchospasm, and atelectasis. Using simultaneously EIT images and MBW we could document changes in mechanical properties of the individual lung associated with changes of ventilation maldistribution. We could further anatomically locate lung areas most affected. It is well known that the acute response to smoke inhalation injury includes airway edema and leukocyte infiltration (13). The release of inflammatory mediators causes bronchoconstriction followed by pulmonary artery constriction, a feature exacerbated by developing oxygen-deficient alveoli. In clinical practice, patients with smoke inhalation injury present with severe peripheral airway obstruction due to endobronchial casts of cell debris. On the basis of studies investigating histological slides of the lung after smoke inhalation, it has been suggested that strategies to remove or decrease formation of airway obstruction may improve respiratory function in victims of smoke inhalation injury (23). The methods presented in our study using noninvasive monitoring may give additional important information to optimize ventilatory support.

**Pulmonary Mechanics**

Overdistension and barotrauma of the lung are most likely to occur after initiation of positive pressure ventilation. As time progresses, smoke inhalation leads to surfactant depletion (5) leading to impaired viscoelastic properties of the lung. Pulmonary function tests before and after smoke inhalation showed early onset of increased airway resistance (severe bronchoconstriction) and decreased lung compliance, which persisted for the study period. This early onset can be explained by smooth muscle contraction only, which is later followed by mucus plugging. The SI 2 h after smoke inhalation was significantly higher. The SI measures during individual tidal breaths whether the compliance of the lung increases or decreases. If the tidal compliance during a breath increases (SI < 1.0) tidal lung recruitment is observed, and if the compliance decreases (SI > 1.0) tidal hyperinflation is present. There is histological evidence that after smoke inhalation alveoli are depleted of functional surfactant (5, 22), resulting in alveolar instability. In daily clinical practice the degree of surfactant depletion can only indirectly be estimated by the measurement of the viscoelastic properties of the lung. However, the interest in surfactant is due solely to its effect on viscoelastic properties; hence the SI is the important, clinically relevant end point, rather than the surfactant per se. The SI is certainly a highly valuable index to assess the degree of surfactant destruction after smoke inhalation injury. It can be obtained in seconds at the bedside without invasive and deleterious bronchoscopies. A secondary aim of this study was to use only pulmonary function tests that are suitable for bedside monitoring. They do not interfere with optimal patient care and allow the treating physician to obtain precise information on anatomical location of lung injury, as well as local changes in response to ven-

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Fig. 8. Example of electrical impedance image before (A) and after smoke inhalation (B). Light areas represent lung regions with good regional ventilation. Ventilation distribution is shifted from the dependent (right) lung to the independent (left) lung.
rior manipulation. Data obtained by such measurements may allow of better titration of ventilatory support in future.

Limitations of the Study

The animals were only observed for 2 h after smoke inhalation, and 2 of 10 animals did not reach a PaO2-to-FIO2 ratio <300. In most experimental and clinical settings smoke inhalation alone rarely causes ARDS (3, 12). Additional burn or bacterial sepsis is an important required concomitant factor for the development of ARDS. Our study focuses on the physiological impact of smoke inhalation alone and does not necessarily reflect a true image of a possible clinical scenario. A longer observation period would be preferable. In this study we have not included any histopathological examinations of the acute lung injury, because we believe that this area has well described in previous studies by Traber’s group (5). We documented high levels of COHb but did not measure other components of the smoke. This has been done previously, and it has been shown that filtering smoke through a 2-μm filter significantly ameliorates the severity of lung injury vs. unfiltered smoke.

In conclusion, we present new data, describing the rapid and regional changes of viscoelastic properties in the lungs after a smoke inhalation and care for the animals subsequently.

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