Effect of poly(ADP ribose) synthetase inhibition on burn and smoke inhalation injury in sheep


POLY(ADP RIBOSE) SYNTHETASE (PARS) is a nuclear enzyme activated by DNA single-strand breaks. This enzyme contains a DNA binding domain, an automodification domain, and a catalytic domain. The DNA binding domain recognizes and rapidly adheres to DNA single-strand breaks. PARS participates in DNA repair in response to genotoxic stress. DNA single-strand breakage is the obligatory trigger of PARS activation, which, in turn, cleaves its substrate nicotinamide adenine dinucleotide (NAD⁺) into nicotinamide and ADP ribose, and covalently attaches ADP ribose units to various nuclear proteins. This process results in a reduced cellular content in NAD⁺, thereby slowing the rate of glycolysis, mitochondrial respiration, and high-energy phosphate generation, and ultimately leading to cell death via the necrotic pathway. In addition, PARS has recently been shown to be involved in the regulation of inflammatory processes, being functionally associated with important transcription factors, notably nuclear factor-κB (NF-κB) (45).

Oxygen- and nitrogen-derived free radicals and oxidants play an important role in the pathogenesis of various forms of trauma. Peroxynitrite, an oxidant produced from the reaction of superoxide and nitric oxide (NO), has been detected in human diseases associated with oxidative stress in acute respiratory distress syndrome (ARDS), endotoxic shock, and pulmonary ischemia and reperfusion injury (39). Reactive oxygen radicals including hydrogen peroxide and hydroxyl radical are additional powerful triggers of DNA single-strand breakage and induce PARS activation (38). Recent studies suggest that PARS has an important role in the pathogenesis of endotoxic shock, hemorrhagic shock, and various types of ischemia-reperfusion injury. Two independent recent studies (7, 22) have demonstrated the role of PARS activation in the pathogenesis of endotoxin and zymosan-activated plasma-induced ARDS in rodents. However, there are no published reports on the effect of PARS inhibition in combined burn and smoke inhalation injury. In the present study, we investigated the effects of a novel potent PARS inhibitor INO-1001 (20) on the pathophysiological changes seen with combined burn and smoke inhalation injury.

MATERIALS AND METHODS

Animals were cared for in the Investigative Intensive Care Unit at our institution, which is approved by the American Association of Laboratory Animal Care. The experimental procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch. The National Institutes of Health and American Physiological Society guidelines for animal care were strictly followed. Animals were studied in the awake state.

INO-1001 was a gift from Inotek Pharmaceuticals ( Beverly, MA). All other reagents used were of analytic grade. Surgical preparation. Eighteen female adult sheep (33.4 ± 1.1 kg (means ± SE)) were surgically prepared for this study.

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All animals were endotracheally intubated and ventilated during the surgery while under ketamine and halothane anesthesia. Arterial catheters (16 gauge, 24 in.; Intracath, Becton Dickinson; Sandy, UT) were placed in the descending aorta via the femoral artery. A Swan-Ganz thermal dilution catheter (model 93A-1317-F, Edwards Critical Care Division; Irvine, CA) was positioned in the pulmonary artery via the right external jugular vein. The chest was opened at the fifth intercostal space on both sides, and an effenter lymphatic from the caudal mediastinal lymph node was cannulated (Silastic medical grade tubing, 0.025 in. ID, 0.047 in. OD, Dow Corning; Midland, MI) by a modification of the technique used by Staub and colleagues (19, 37). The systemic contributions to the node were removed by ligation of the tail of the caudal mediastinal lymph node and cauterization of the systemic diaphragmatic lymph vessels (8). Through the fifth intercostal space, a catheter (Durastic Silicone Tubing DT08, 0.062 in. ID, 0.125 in. OD, Allied Biomedical) was positioned in the left atrium. The sheep were given 5–7 days to recover from the surgical procedure and given free access to food and water.

Bacterial infection: Inhalation injury. Before the injury was produced, after induction of anesthesia with the use of 10 mg/kg ketamine (Ketalar; Parke-Davis; Morris Plains, NJ), a Foley catheter was placed in the urinary bladder to determine urine output. The animals then received a tracheotomy, and auffed tracheostomy tube (10-mm diameter, Shiley; Irvine, CA) was inserted. The anesthesia was then continued with halothane. After the wool had been shaved off the animal, a 20% total body surface area third-degree flame burn was made on one flank. The burn was produced with a Bunsen burner that was applied until the skin was thoroughly contracted. We have previously determined this degree of injury to be a full-thickness burn, i.e., including both epidermis and dermis, in which the nerve endings are destroyed by heat. Therefore, inhalation injury was induced while the sheep was in the prone position as previously described (19, 32). A modified bee smoker was filled with 50 g of burning cotton toweling and was connected to the tracheostomy tube via a modified endotracheal tube containing an indwelling thermistor from a Swan-Ganz catheter. During the insufflation procedure, the temperature of the smoke did not exceed 40°C. The sheep were insufflated with a total of 48 breaths of cotton smoke. After smoke insufflation, another 20% total body surface area third-degree burn was made on the contralateral flank.

Resuscitation protocol. Immediately after injury, anesthesia was discontinued and the animals were allowed to awaken but were mechanically ventilated with a Servo ventilator (model 900C, Siemens-Elena; Solna, Sweden) throughout the next 72-h experimental period. Ventilation was performed with a positive end-expiratory pressure of 5 cmH2O and a tidal volume of 15 ml/kg. The respiratory rate was set to maintain normocapnia; thereafter, it was adjusted to maintain the arterial oxygen saturation >90%. These respiratory settings allowed a rapid disappearance of carboxyhemoglobin after smoke inhalation.

Fluid resuscitation during the experiment was performed with Ringer’s lactate solution following the Parkland formula (4 ml·% burned surface area·kg body wt−1 per the first 24 h and 2 ml·% burned surface area·kg body wt−1 per day for the next 48 h) (1). The Parkland formula was begun 1 h after injury. One-half of the volume for the first day was infused in the initial 8 h, and the rest was infused in the next 16 h. Urine was collected and urine output was recorded every 6 h. Fluid balance was determined by total fluid volume infused minus urine output and was reported as milligrams per kilogram per day. During this experimental period, the animals were allowed free access to food, but not to water, to allow accurate determination of fluid balance.

The sham animals were all ventilated in the same manner and were given the same amount of the fluid as the injured sham group.

Measured variables. Measured physiological parameters were not considered valid until the animals were fully awake and standing, usually within 1 h of injury. Mean arterial pressure (MAP; in mmHg), pulmonary arterial pressure (PAP; in mmHg), left atrial pressure (in mmHg), and central venous pressure (in mmHg) were measured with pressure transducers (model PX-1800, Baxter, Edwards Critical Care Division) that were adapted with a continuous flushing device. The transducers were connected to a hemodynamic monitor (model 78304A; Hewlett-Packard; Santa Clara, CA). Zero calibrations were taken at the level of the olecranon joint on the front leg, which is considered to be the level of the right atrium. Cardiac output was measured with a cardiac output computer (CO Computer DM-701, Baxter Critical Care Division) by the thermodilution method with 5% dextrose as an indicator solution. For evaluation of cardiac function, cardiac index (in l·min−1·m−2), left ventricular stroke work index (in g·min−1·m−2), and pulmonary resistance (PVR; in dyn·s·cm−5·m−2) were calculated with standard equations. Arterial and mixed venous blood was measured with a blood gas analyzer (model IL1600; Instrumentation Laboratory; Lexington, MA). The data were corrected for the body temperature of the sheep. Oxymoglobin saturation and carboxyhemoglobin concentration were analyzed with a COoximeter (model IL482; Instrumentation Laboratory). Hematocrit was measured in heparinized microhematocrit tubes (Fisherbrand, Fisher; Pittsburgh, PA). Lung lymph flow was measured with a graduated test tube and stopwatch. Lymph and blood samples were collected in EDTA tubes, and the total protein concentration in plasma (Cp) and lymph (C L) were measured with a refractometer (National Instrument; Baltimore, MD). Thereafter pulmonary microvascular permeability index (PI) was calculated by the following equation: PI = Q L · (C L/C p), where Q L is lung lymph flow (ml/h). Shunt fraction was calculated using standard equations. The colloid oncotic pressures in plasma and lung lymph were determined through a semipermeable membrane in a colloid osmometer (model 4100, Wescor; Logan, UT). Nitrite/nitrate (NOx; total amount of NO metabolites) in plasma and was measured with an NO chemiluminescence detector (model 7025, Antek Instruments; Houston, TX).

Measurement of tracheal blood flow. To determine the tracheal blood flow, fluorescent microspheres (12 × 106, Interactive Medical Technologies; West Los Angeles, CA) were injected into the left atrium at different time points. Immediately after injection of microspheres, blood was withdrawn (model 55-1143, Harvard Apparatus; South Natick, MA) from the femoral artery at the rate of 10 ml/min; withdrawal was started right after microsphere injection and continued for 2 min. Tissue samples of trachea were obtained postmortem and used to quantify tracheal blood flow.

Measurement of lung wet-to-dry weight ratio and histological analysis. Forty-eight hours after the insult, the animals were killed, and lung tissues were taken for measurement of lung wet-to-dry weight ratio (28) and histological analysis. Lung tissue was obtained for histopathological examination after a standardized sampling protocol. A 1-cm-thick midline vertical slice through the lower lobe of the right lung was injected with 10% buffered formalin, followed by immersion.
in fixative for 3–5 days. After fixation, the tissue slice was sampled at three standard sites by a technician unaware of the treatment group. The tissue was embedded in paraplast, sectioned at 4 μm, and stained with hematoxylin and eosin. All slides obtained were then coded to allow masked assessment of parenchymal histopathology and airway obstruction. Histopathologic assessment consisted of assigning a semi-quantitative score for degrees of congestion, edema, inflammation, and hemorrhage for 24 individual ×10 objective fields from the three slides from each animal. The semiquantitative scores were as follows: 0 = apparently normal, 1 = mild abnormality, 2 = moderate, 3 = strong, and 4 = intense and widespread abnormality. After the 24 individual assessments for each category were completed, an overall mean score was determined for each animal. The mean degree of airway obstruction was determined by the following protocol. All cross-sectioned airways were identified in each slide and classified as bronchi, bronchioles, or terminal/respiratory bronchioles. Bronchi had associated mucous glands and/or cartilage, bronchioles lacked mucous glands and cartilage, and terminal/respiratory bronchioles had short cuboidal lining epithelial cells and lacked surrounding smooth muscle tissue. For each airway, a degree of lumenal obstruction was estimated from 0 to 100%. From these data, mean degrees of bronchial, bronchiolar, and terminal/respiratory bronchiolar obstruction were determined for each animal.

**Lung myeloperoxidase activity.** The activity of myeloperoxidase, an indicator of neutrophil accumulation, was determined directly in whole lung homogenates. Pieces of lung tissue or an aliquot of the homogenate were mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM hydrogen peroxide. Activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37°C, using a Spectramax microplate reader (Molecular Devices; Sunnyvale, CA). Results are expressed as milliunits of myeloperoxidase activity per milligram tissue protein.

**Malondialdehyde formation in lung homogenates.** Malondialdehyde formation was utilized to quantify the lipid peroxidation in the lung and measured as thiobarbituric acid-reactive material. Lung samples were homogenized (50 mg/ml) in 1.15% KCl buffer. Homogenates (200 μl) were then added to a reaction mixture consisting of 1.5 ml 0.8% thiobarbituric acid, 200 μl 0.1% sodium dodecyl sulfate, 1.5 ml 20% acetic acid (pH 3.5), and 600 μl distilled H2O and heated at 90°C for 45 min. After being cooled to room temperature, the samples were cleared by centrifugation (10,000 g, 10 min) and their absorbance was measured at 532 nm, using 1,1,3,3-tetramethoxypropane as an external standard. The level of lipid peroxides was expressed as nanomoles MDA per milligram protein, measured by the Bradford assay.

**Poly(ADP ribose) immunohistochemistry.** To confirm the PARS inhibitory effect of INO-1001, PARS activity in tissues was measured by using an immunohistochemical method of PARS activity utilizing biotinylated NAD (1). Briefly, cryosections (10 μm) were fixed for 10 min in 95% ethanol at 20°C and then rinsed in PBS. Sections were permeabilized by 1% Triton X-100 in 100 mM Tris pH 8.0, for 15 min. Reaction mixture (10 mM MgCl2, 1 mM dithiotreitol, 30 mM biotinylated NAD+, in 100 mM Tris, pH 8.0) was then applied to the sections for 30 min at 37°C. Reaction mixes containing 5 mM 3-aminobenzamide or biotinyl-NAD+ free reaction mix were used as controls. After three washes in PBS, incorporated biotin was detected by peroxidase-conjugated streptavidine (1:100, 30 min, room temperature). After ~3–10 min washes in PBS, color was developed with cobalt-enhanced nickel diaminobenzidine (DAB) substrate (13). Sections were counterstained in Nuclear Fast Red, dehydrated, and mounted in Vectamount.

**Experimental protocol.** The animals were randomized into three groups as follows: a PARS inhibitor-treated group given bolus intravenous injection of 3 mg/kg INO-1001 at 1 h post-injury, followed by a 0.3 mg·kg−1·h−1 continuous infusion for 48 h (n = 6); a vehicle-treated injured control group (given distilled water in the same fashion as for INO-1001; n = 6); and a sham injury group (no injury, no treatment; n = 6).

Lymph and blood samples for determination of total protein concentration, colloid osmotic pressure, and NOX were collected from all three groups of animals in our study at 3, 6, 12, 18, 24, 30, 36, and 48 h after injury. Hemodynamic variables and blood gases were measured at 3, 6, 12, 18, 24, 30, 36, 42, and 48 h after injury in all groups. When all measurements were completed, animals were anesthetized with ketamine and humanely euthanized by administration of a saturated potassium chloride solution. Immediately after death, the right lung was used for pathological examination and the left lung was used for measurement of wet/dry weight ratio, corrected for the content of blood, as described by Pearce and colleagues (28).

**Statistical methods.** All values are expressed as means ± SE. Outcome variables for physiological parameters were analyzed using analysis of variance for a two-factor experiment with repeated measures over time. The two factors are experimental groups and time. Fisher’s least-significant difference procedure was used for multiple comparisons (or post hoc statistical analysis). For the histological study, a non-parametric Kruskal-Wallis test was performed and Mann-Whitney’s U test was used to compare data within the groups. Measurements at various time periods were tested at the 0.05 level of significance.

### RESULTS

All animals in all groups survived during the 48-h experimental period. The arterial carboxyhemoglobin levels, as measured immediately after smoke exposure, amounted to 60.1 ± 4.0% in the vehicle-treated group and 56.1 ± 4.8% in the PARS inhibitor-treated group. There was no statistically significant difference between these two values.

**Lung wet/dry weight ratio.** The blood-free wet/dry weight ratio of the right lung was significantly higher in the injured control group than in the sham group (6.23 ± 0.33 in the injured control group vs. 5.22 ± 0.07 in the sham group; P < 0.05) (Fig. 1). The PARS inhibitor-treated group showed significantly lower values than the injured control group (5.42 ± 0.08 in the PARS inhibitor group vs. 6.23 ± 0.33 in the injured control group; P < 0.05).

**Pulmonary transvascular fluid flux.** Pulmonary transvascular fluid flux was evaluated by measurement of lung lymph flow and the pulmonary permeability index. The injured control group showed significant increases in both lung lymph flow and pulmonary permeability index from the sham group. This increase was significantly attenuated by treatment with the PARS inhibitor (Fig. 2, A and B).

**Gas exchange and pulmonary shunt fraction.** The injured control group showed a progressive fall in PaO2/FiO2 ratio, which was significantly lower than the baseline value 12 h after injury. During the entire experi-
mental period, the PaO2/FIO2 ratio of the PARS inhibitor-treated group remained above that of the injured control group. There were significant differences in PaO2/FIO2 ratio between the PARS inhibitor and injured control groups beyond 30 h after injury (Fig. 3A). The intrapulmonary shunt fraction significantly increased in the injured control group. The increase was significantly less in the PARS inhibitor group (Fig. 3B).

**Histopathological examination.** PARS inhibitor treatment reduced the obstruction scores significantly for bronchi and respiratory bronchioles (Fig. 4A). PARS inhibitor treatment significantly reduced the total histological scores for edema, congestion, inflammation, and hemorrhage (Fig. 4B). Typical histopathological changes are shown in Fig. 5.

**Airway pressure.** In the control group, both peak and pause airway pressures increased significantly after injury. Treatment with the PARS inhibitor attenuated these changes (Fig. 6, A and B).

**Airway blood flow.** The tracheal blood flow in the burn and smoke group dramatically increased after injury (2,590 ± 222% of the baseline value in the injured control burn and smoke group 3 h after injury; P < 0.05 compared with the sham group). The blood flow values in the PARS inhibitor group showed a lesser degree of increase (1,060 ± 336% of the baseline value 3 h after injury) (Fig. 7). The airway blood flow consists of two blood supplies, the systemic and pulmonary circulations. The lower trachea harvested in the present study was supplied by the bronchial arteries from the systemic circulation (6).

**Pulmonary hemokinetics.** In the control group, PAP increased rapidly after injury and remained elevated...
significantly for 48 h. Treatment with the PARS inhibitor attenuated this change. The pulmonary vascular resistance index (PVRI) showed changes parallel to the PAP (Table 1).

**Urine output and hematocrit.** The urine output was significantly higher in the PARS inhibitor group than in the control group beyond 30 h after injury (Fig. 8A). The control group showed a significant increase in hematocrit after injury (25.7 ± 0.3% of baseline, 30.3 ± 2.1 of 48 h; \( P < 0.05 \)), whereas the PARS inhibitor-treated group showed no significant changes after injury (27.2 ± 1.6% at baseline, 27.6 ± 2.7 at 48 h; \( P < 0.05 \)) (Fig. 7B).

**Cardiopulmonary hemodynamics.** Table 1 presents cardiovascular variables measured during the experiment. MAP remained unchanged in both groups. Cardiac index (cardiac output/body surface area) remained unchanged in the injured control group. However, in the PARS inhibitor-treated group, the cardiac index increased significantly from baseline. In the control group, the left ventricular stroke work index decreased significantly 18 h after the insult but was maintained in the PARS inhibitor-treated group.

**Plasma NOx.** In the control group, the NOx levels in plasma increased after injury and remained significantly elevated for 48 h. In the PARS inhibitor-treated group, NOx levels increased initially but gradually decreased after 12 h (Fig. 9).
Lung malondialdehyde formation of the right lung was significantly higher in the injured control group than in the sham group (556 ± 41.5 nmol/mg protein in the injured control group vs. 218.3 ± 62.8 nmol/mg protein in the sham group; P < 0.01) (Fig. 10). The PARS inhibitor-treated group showed significantly lower values than the injured control group (357 ± 44.4 nmol/mg protein in the PARS inhibitor group vs. 556 ± 41.5 nmol/mg protein in the injured control group; P < 0.05).

Malondialdehyde formation in lung homogenates. Lung malondialdehyde formation of the right lung was significantly higher in the injured control group than in the sham group (8.3 ± 0.3 in the injured control group vs. 5.6 ± 0.4 nmol/mg protein in the sham group; P < 0.05) (Fig. 11). The PARS inhibitor-treated group showed significantly lower values than the injured control group (6.6 ± 0.4 nmol/mg protein in the PARS inhibitor group vs. 8.3 ± 0.3 nmol/mg protein in the injured control group; P < 0.05).

Lung immunohistochemistry. After smoke/burn injury, there was a marked activation of PARS in the lung, as determined by an immunohistochemical method that detects the accumulation of poly(ADP ribose), the product of PARS enzyme in the tissues. There was a marked increase in poly(ADP ribose) immunoreactivity in the injured lung areas, localizing mainly to the pulmonary epithelium and to the infiltrating mononuclear cells. This activation was abolished in the INO-1001-treated animals, which also exerted a marked improvement in the overall histological picture (Fig. 12).

All of the variables for the sham group and the baseline values for the injured groups were within normal limits. The values for the sham group remained at baseline levels for the 48-h time period of study. They did not demonstrate any gross or micropathological changes in their lung tissues at autopsy.

**DISCUSSION**

There are many published studies demonstrating that PARS activation plays an important role in the pathogenesis of endotoxic shock, inflammation, and ischemia-reperfusion injury (27, 38, 45). INO-1001 is a novel and potent PARS inhibitor, which has recently been shown to be effective in rodent models of stroke (20). Another potent PARS inhibitor, PJ34, has recently been synthesized and characterized (17, 22, 41). INO-1001 is ∼10–30 times more potent PARS inhibitor than PJ34. On the isolated enzyme, the inhibition constant is 15 nM. As we showed in the immunohistochemical history study (Fig. 12), INO-1001 markedly inhibited the poly(ADP ribose) formation after burn and smoke/burn injury.

**Table 1. Hemodynamic changes**

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Values are means ± SE. PAP, pulmonary arterial pressure; PVRI, pulmonary vascular resistance index; MAP, mean arterial pressure; CI, cardiac index; LVSWI, left ventricular stroke work index. *P < 0.05, significant difference from control; †P < 0.05, significant difference from sham.
smoke injury. The structure of INO-1001 will be disclosed soon.

NO produced by NO synthase (NOS) plays a major role in the pathogenesis of trauma and sepsis (11, 23).

Large amounts of superoxide anions are known to be present in burn/smoke and are inactivated within a few minutes. Several studies on burn/smoke inhalation injury have demonstrated that lipid peroxides appear in the systemic circulation within minutes after injury and peak a second time at 24 h after injury (21). Peroxynitrite is a potent oxidant produced by the reaction of NO and superoxide anion (3, 44) and is produced in burn and smoke models of injury (33). In another pathway, singlet dioxygen is produced in burn and smoke injury (18). Singlet dioxygen and peroxynitrite induce the development of DNA single-strand breakage with resulting PARP activation. Intracellular NAD$^+$ and ATP levels are depleted as a consequence of PARP activation. Depletion of cellular NAD leads to inhibition of ATP generating pathways, which leads to cellular dysfunction. In addition, it was recently reported that PARP activates NF-$\kappa$B and thereby the expression of the inducible isoform of NO synthase.

Fig. 8. A: urine output (ml·h$^{-1}$·kg$^{-1}$). Data are expressed as the means ± SE. Gray bars represent the sham group ($n = 6$), open bars represent the injured control group ($n = 6$), and solid bars represent the PARS inhibitor group ($n = 6$). B: hematocrit (%). Data are expressed as means ± SE. ●, Sham group ($n = 6$); ■, injured control group ($n = 6$); ▲, PARS inhibitor group ($n = 6$). BL, baseline. *$P < 0.05$ vs. control; †$P < 0.05$ vs. sham.

Fig. 9. Nitrate/nitrite (NOx) levels in plasma (% of baseline values). Data are expressed as means ± SE. ●, Sham group ($n = 6$); ■, injured control group ($n = 6$); ▲, PARS inhibitor group ($n = 6$). BL, baseline. *$P < 0.05$ vs. control; †$P < 0.05$ vs. sham.

Fig. 10. Lung myeloperoxidase (MPO) activity in sham ($n = 6$), control with burn/smoke ($n = 6$), and PARS inhibitor-treated groups ($n = 6$). Data are expressed as means ± SE. *$P < 0.05$ vs. control; †$P < 0.01$ vs. sham group.

Fig. 11. Lung malondialdehyde (MDA) formation in sham ($n = 6$), control with burn/smoke ($n = 6$), and PARS inhibitor-treated groups ($n = 6$). Data are expressed as means ± SE. *$P < 0.05$ vs. control; †$P < 0.01$ vs. sham group.
(iNOS) as well as the production of various proinflammatory cytokine and chemokine mediators (13, 15, 40). In this study, in accordance with our previous reports, we found that there was an elevation of NOx in this model. Also, we (35) have previously demonstrated that there is an elevation in nitrotyrosine immunoreactivity, an index of reactive nitrogen species generation (such as peroxynitrite formation). In our prior work (35), we demonstrated that the elevation of NOx and the formation of nitrotyrosine were prevented by administration of an iNOS inhibitor. In the present study, plasma NOx levels were suppressed by the treatment with the PARS inhibitor. It is conceivable that this effect was related to the inhibition of PARS activation of the NF-κB pathway. In the current study, we used high tidal volume setting because severe respiratory acidosis was frequently observed in lower tidal and atelectasis volume setting and sodium bicarbonate was needed to normalize pH, and sometimes we needed to sedate the animal because of ethical problems. We confirmed that a high tidal volume setting (15 mg/kg) does not harm the lung for at least 48 h. However, airway obstruction may cause an increase in airway pressure and barotraumas. In the present study, INO-1001 attenuated the increase in airway pressure, which may be one of the mechanisms of action by which it improved acute lung injury after burn and smoke. A recent study (42) recommended low tidal volume ventilation (6 mg/kg). However, there are some critiques on this concern (26). In our ovine model of acute lung injury, the lower tidal ventilation was not good. Therefore, we used the high tidal volume setting.

Demling et al. (9) reported that pulmonary microvascular permeability and vascular permeability in unburned soft tissue increased after a major cutaneous burn. We (25, 31) have reported that smoke inhalation will likewise increase systemic transvascular fluid flux with and without burn injury. The vascular leakage releases a large amount of fluid into the interstitial spaces. The consequent edema formation is more severe when thermal injury is associated with smoke inhalation injury. Recent studies have focused on the role of NO in vascular hyperpermeability seen with sepsis or burn. Soejima et al. (34) administered mercaptoethylguanidine, a selective iNOS inhibitor, in the ovine smoke/burn model and demonstrated its effectiveness in acute lung injury. mercaptoethylguanidine attenuated lung edema formation. We have obtained similar findings with another potent specific iNOS inhibitor, BBS-2 (Berlex) in our burn and smoke inhalation model. In preliminary studies, administration of BBS-2 increased urinary output, normalized fluid balance and hematocrit, and increased cardiac output (10). In the current study, a PARS inhibitor attenuated the pulmonary permeability index and reduced the pulmonary wet/dry weight ratio significantly. As a result, in the PARS inhibitor group, urine output was significantly higher than in the injured control group beyond 30 h after injury.

In the injured control group, PAP and pulmonary vascular resistance index (PVRI) rapidly increased after injury and remained elevated significantly for 48 h. However, in the PARS inhibitor-treated group, both PAP and PVRI remained nearer to the baseline level. The current experimental model is characterized by progressive and sustained pulmonary hypertension. The progressive increase in PVRI is likely a result of serial inflammatory events in the pulmonary microvascularity. Because all of the animals were mechanically ventilated, an increase in airway pressures due to airway obstruction may contribute to the increase in PAP and PVRI. The PARS inhibitor’s beneficial effects on airway obstruction and airway pressures could account for attenuation of PAP and PVRI. The exact mechanisms responsible for the changes in lung mechanics in ARDS or septic shock remain unclear. It has been proposed that augmentation of pulmonary resistance is caused by a narrowing of airway lumens as a
result of epithelial and interstitial edema, as well as by active airway smooth muscle contraction caused by local release of inflammatory mediators such as thromboxane and leukotrienes (14, 24, 29). The increase in these inflammatory mediators contributes to airway edema and formation of exudates that coagulate to form casts. The current study demonstrates that the PARS inhibitor reduced both the morphological and the physiological changes in the lung. As in other complex models of shock, inflammation, and tissue degeneration, these beneficial actions are likely to be due to a combination of INO-1001’s multiple actions, including reduction of iNOS expression, downregulation of inflammatory mediator production, and inhibition of cell injury produced by energy depletion and oxidative damage.

In the present study, airway blood flow was measured in both injured groups. The control group showed a significant increase in blood flow shortly after injury. The PARS inhibitor attenuated this early change. The augmented airway blood flow, associated with inflammation in the airway, contributes to the development of lung tissue damage seen with smoke inhalation injury by carrying inflammatory mediators from the injured airway to the parenchyma through the bronchopulmonary venous drainage, which is a communication between the systemic circulation supplying the airways and the pulmonary circulation (16, 30). We have previously reported that the increase in bronchial blood flow was reduced by the administration of an iNOS inhibitor (10). We also have demonstrated the presence of nitrotyrosine, an indicator of peroxynitrite formation in the airway (35). It is conceivable that the protection against the airway blood flow changes seen with PARS inhibition is related to the prevention of NO overproduction in the current experimental model.

From the present data, we conclude that in a clinically relevant model of burn- and smoke inhalation-induced acute lung injury, the PARS inhibitor INO-1001 attenuates: 1) lung edema formation, 2) abnormalities in gas exchange, 3) elevated airway blood flow, 4) increased airway resistance, 5) lung histopathological changes, and 6) systemic vascular leakage. These findings significantly advance our understanding with respect to the pathogenesis and experimental therapy of acute lung injury. Previous studies (7, 22) focusing on PARS and ARDS have utilized murine models, stimuli other than smoke and burn (endoxin and zymosan-induced plasma, respectively), induced pathophysiological alterations that were mainly confined to the lung, and measured only a limited number of hemodynamic and physiological variables. The present study confirms and expands the prior findings and indicates that PARS activation plays a central role in the pathogenesis of smoke- and burn-induced pulmonary and systemic pathophysiological alterations. The ovine preparation used in the current study adequately models most aspects of human ARDS and has proven to be useful in testing novel experimental therapeutic interventions (4, 35). For that reason, the positive benefit of PARS inhibition seen in the present study may suggest potential utility of PARS inhibitors in the management of human patients with burn and smoke inhalation injury.

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REFERENCES


