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A murine model of smoke inhalation

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SMOKE INHALATION is the leading cause of death in victims of structural fires (8). Although major advances have been made in the treatment of burns, advances in the treatment of smoke inhalation injury have been limited (10). Smoke inhalation injury can be characterized according to the time period postinjury. The first 36 h involves treatment of the initial hypoxic insult, carbon monoxide and cyanide toxicity, early airway edema, bronchoconstriction, mucosal sloughing, tracheobronchitis, increased lung water, and impaired gas exchange characterize days 1–5 postexposure. The final stage is the inflammation-infection stage, in which the risk of nosocomial pneumonia increases markedly, coinciding with further impairment of lung function (8).

As observed in human victims, animal models of smoke inhalation injury characterized by other investigators demonstrate not only mortality (23) but also lung tissue damage (18, 25, 26, 28), elevation of inflammatory mediators (1, 9), and the deleterious effects of the inhalation of noxious gases (8, 29, 33). Sheep (25, 30), dogs (5), and rabbits (21) are among the best characterized of these animal models. Despite the valuable information generated through these studies, the current understanding of the mechanisms behind the observed pathophysiology of smoke inhalation damage is limited. To dissect the pathways involved in smoke inhalation damage, a molecular understanding is necessary. Because of the availability of inbred strains and the ability to target specific genes and genetically target specific cell types (12, 31), the mouse may prove to be an excellent experimental model in which to evaluate and develop treatment strategies for lung injury. Therefore, a mouse model for smoke inhalation has been established through the use of a novel murine smoke chamber and subsequent determination of the dose-death relationship, percent carboxyhemoglobin (COHb), lung pathology, bronchoalveolar lavage (BAL) fluid cell count, and tumor necrosis factor (TNF)-α levels. This model reflects the carbon monoxide-induced mortality and tissue damage observed in human patients.

MATERIALS AND METHODS

Smoke chamber. The smoke chamber (Fig. 1A) consists of a power supply, an incinerator (Fig. 1B), a circulation fan, and an inhalation chamber. The power supply, with an adjustable voltage source, controls the DC current through a series circuit to the resistive element within the incinerator. Not only is the amount of heat generated regulated by the adjustable power supply, but the incinerator also has a tempered glass door for visual monitoring of the rate of combustion. The resistive element consists of removable nichrome wire that is coiled tightly (Fig. 1B) to allow more heat per area than an uncoiled wire. A circulation fan (Minispiral AC, EG & G Rotron, Saugerties, NY) with adjustable speed is connected to the inlet of the incinerator and circulates the smoke through the inhalation chamber (3.48 liters) in this closed system (4.08 liters). The inhalation chamber containing the mice consists of a Plexiglas cylinder with removable nichrome wire that is coiled tightly below the wire mesh. A CD-1 model of wood smoke exposure was developed, demonstrating type II cell hypertrophy, or polyurethane smoke. A CD-1 model of wood smoke exposure was developed, demonstrating type II cell hypertrophy, or polyurethane smoke.
(100 μg/g body wt), xylazine (5 μg/g body wt), and acepromazine (2.5 μg/g body wt) and placed in pairs into the smoke chamber (Fig. 1A) for defined periods of wood smoke exposure. Untreated pine lumber (12 3 13 4 inches) was cut into small uniform rectangular pieces (20 3 3 3 mm) weighing a total of 0.1–0.5 g and placed between the heating coils in the incinerator (Fig. 1B). The wood was burned slowly so that it would not produce a flame. As the incinerator filled with smoke, the fan was turned on to circulate the smoke into the chamber with the anesthetized mice. After a defined period, the mice were removed, and the survivors were allowed to awaken from the anesthesia (Fig. 2). Control mice were also anesthetized and placed in the smoke chamber with intermittent exposure to air circulated by the fan but no smoke. In order to determine which material produced the greatest lung damage 48 or 72 h after a 12- to 15-min exposure, initial studies involved the pyrolysis of 10 mg/kg body wt of cotton, polyurethane, and wood (hardwood) to which female Swiss albino inbred mice (FVB/n, Jackson Laboratories) anesthetized with chloral hydrate (0.1 ml/g body wt) were exposed.

Dose-mortality studies. Mice were placed in the inhalation chamber for 20 min and exposed to smoke from the smoldering of increasing quantities of pine wood (3–17 mg/g body wt). Mice were observed for at least 7 days and then killed. No other parameters were measured in these mice.

COHb measurements. Immediately after removal from the smoke chamber, blood was collected in a heparinized tube from the tail vein of each mouse. COHb levels were measured spectrophotometrically with a CIBA Corning Co-ox 270 meter. Samples (~50 μl) were collected every hour postexposure until COHb levels returned to normal.

Lung pathology. Lungs were infused via the airway with 1.4 ml of 10% formalin at 25-cm fluid height (the distance between the lungs and the meniscus of the formalin in the reservoir), embedded in paraffin, sectioned (4 mm), and stained with hematoxylin and eosin to examine the pathology.

BAL fluid cell count. BAL was performed as described in Lung pathology 48 h after smoke exposure. Total cell counts were performed with a hemocytometer and Gentian violet stain.

TNF-α measurements. Mice were anesthetized and exsanguinated. The diaphragm was cut, the chest was opened, and a blunt-end catheter was inserted into the trachea. Three 1-ml aliquots of Hanks’ balanced salt solution (GIBCO BRL, Life Technologies, Grand Island, NY) were instilled into the lungs and removed three times, each with a 1-ml syringe. The supernatant from the first milliliter of BAL fluid was frozen at −70°C. The cells from all three aliquots were pooled, resuspended in RPMI 1640 medium (GIBCO BRL) with 10% FBS and 1% penicillin-streptomycin, and used for 22-h cell culture at 37°C in 5% CO₂ with 1 μg/ml of lipopolysaccharide (LPS). The supernatant of the cultured cells was removed and frozen at −70°C. TNF-α levels were determined for both the initial lavage supernatant and postculture supernatant with an ELISA kit (BioSource, Camarillo, CA).

Static lung compliance measurements. Treated and control mice were injected with a lethal dose of pentobarbital sodium (Abbott Laboratories, North Chicago, IL) and placed in a container containing 100% oxygen to ensure complete collapse of the alveoli by oxygen absorption before death. The tracheae were cannulated and connected to a syringe and pressure transducer (X-ducer, Motorola, Phoenix, AZ) via a three-way connector. After the diaphragm was opened, lungs were inflated in 100-μl increments every 5 s to a maximum inflation pressure of 30 cmH₂O and then sequentially deflated. Pressure and volume on inflation and deflation were measured.
recorded. Pressure-volume curves were generated for each animal. Lung compliance was determined by calculating the slope of the linear portion of the deflation curve between +10 and −10 cmH₂O.

Statistical analysis. All statistical comparisons were made by one-way ANOVA. P values < 0.05 were considered significant. These analyses were followed up by Student-Newman-Keuls tests in which P values < 0.05 were considered significant.

RESULTS

Dose-mortality studies. Mice were exposed to 20 min of smoke from the combustion of varying amounts of pine (mg wood/g body wt). Values are means ± SD. All the mice that received smoke from the combustion of smaller amounts (≤5 mg/g of wood) survived the exposure, but with increasing dose, the percentage of mortality increased (0–24 h postexposure) to a 100% lethal dose (≥13 mg/g of wood; Fig. 3).

COHb measurements. The mean percent COHb of control mice was 0.2 ± 0.05%. The mean percent COHb of mice (n = 13) exposed to wood smoke (10.0 mg wood/g body wt) for 20 min was 50.1 ± 2.0%. The blood level of COHb of mice that died from smoke exposure was 77.6 ± 6.0%. Percent COHb levels were measured every hour in the mice from the sublethal smoke group until values were comparable to control values. The time needed for half the carbon monoxide bound to hemoglobin to be released was ~60 min (Fig. 4).

Lung pathology. The lungs of mice exposed to wood, cotton, and polyurethane smoke were fixed in 10% formalin 48 h after exposure. The distal lungs of mice exposed to wood smoke demonstrated cell death as evidenced by cytoplasmic vacuolization, cytoplasmic blebbing, and severe sloughing of the bronchiolar epithelium (Fig. 5, Table 1). Furthermore, peracute hemorrhage, capillary congestion, and peribronchiolar lymphocytic cuffing were present. In the proximal airways of these mice, cytoplasmic vacuolization of the tracheal epithelium (score = 2.5) was observed, along with cytoplasmic blebbing (score = 1) and cytoplasmic vacuolization (score = 4.5) of the epithelium of the main-stem bronchi (Fig. 5, Table 1). The distal lung pathology observed after cotton smoke inhalation included cytoplasmic blebbing, cytoplasmic vacuolization of the bronchiolar epithelium, very mild type II cell hypertrophy, and very mild neutrophil influx as well as cytoplasmic vacuolization (score = 2.5) and sloughing (score = 1.5) in the proximal lung (Table 1). Polyurethane smoke also produced cytoplasmic vacuolization, cytoplasmic blebbing, and hyperplasia in the distal lung as well as tracheal vacuolization (score = 2.5).

Because the most severe pathology was observed after wood smoke exposure, wood smoke was used in the subsequent development of a CD-1 mouse model of smoke inhalation. As in the FVB/n mice, these mice demonstrated cytoplasmic blebbing, cytoplasmic vacuolization, and sloughing of the bronchiolar epithelium as well as perivascular edema, peracute hemorrhage, alveolar histiocytosis, lymphocytic influx into the alveoli, type II cell hypertrophy (Fig. 6, Table 1) and type I cell hyperplasia (Table 1) at 48 (or 24 h postexposure to 7–9 mg/g of wood smoke in the distal lung as well as cytoplasmic blebbing (score = 2.5), vacuolization, and sloughing in the proximal lung (Fig. 7). It is important to note that any score above 0 (normal lung) is significant after such an acute smoke exposure. A score of 5 would only be expected in a severely diseased lung and would usually require a longer-term exposure to such injurious agents.

BAL fluid cell counts. The lungs of mice (Fig. 8) exposed to smoke demonstrated a 2.8-fold increase over control values in the mean total cell count.

TNF-α levels. The mean TNF-α levels in the BAL fluid supernatant of mice 48 h after smoke exposure (10 mg/g body wt) were not significantly elevated. Elvation of the supernatant TNF-α level of BAL fluid cells cultured for 22 h in 1 μg/ml of LPS indicates that these cells were competent to release TNF-α (Fig. 9). LPS is found on gram-negative bacteria and has been demonstrated to stimulate TNF-α release from alveolar macrophages (24).
Static lung compliance measurements. Static lung compliance values were determined from the linear portion of the deflation curve between −10 and +10 cmH₂O. The mean static lung compliance in mice exposed to varying concentrations of wood smoke 24, 48, and 72 h postexposure were not significantly different from control values (Fig. 10).

DISCUSSION

Through the implementation of a murine smoke chamber designed and constructed in this laboratory, a murine model of smoke injury has been developed. This model system was used to determine the relationship between dose and mortality as well as to evaluate lung tissue damage and carbon monoxide poisoning. This murine model reflects the injury and mortality seen in humans in that carbon monoxide-stimulated mortality was observed in addition to lung tissue damage. Furthermore, the nature of the lung tissue damage and the COHb levels immediately after exposure were similar to those observed in humans (27).

Fires and the smoke they produce are the most common source of carbon monoxide poisoning (16). Wood smoke in particular contains large quantities of carbon monoxide (7, 33). Tissue hypoxia is the most important effect of carbon monoxide (2, 16). The most reliable index of the amount of carbon monoxide in the body is the percentage of COHb (11). Nonsmokers have COHb levels of <3% (17). Mild cases of human carbon monoxide poisoning (<30% COHb) are usually accompanied by headaches, nausea, vomiting, and dizziness but no cardiovascular or neurological symptoms. Patients who do not show cardiovascular or neurological symptoms but have COHb levels between 30 and 40% are considered moderate cases that need to be monitored should symptoms arise (16). As carbon monoxide poisoning becomes severe (>40%), patients show tachyphagia, tachypnea, neurological symptoms, and even coma. When blood COHb levels reach 40–50%, headache, confusion, and collapse may occur (32). A COHb level of 60–70% causes unconsciousness, intermittent convulsions, respiratory failure, and death if exposure continues (32). All patients are given 100% oxygen but the more severe cases involving coma may
require hyperbaric oxygen. The percent COHb levels observed in our mice immediately after smoke exposure were comparable to the levels in humans for nonexposed and sublethal and lethal exposures.

The association between mortality and carbon monoxide poisoning was made as a result of both the time of death and blood levels of COHb. Mortality from an inflammatory response and lung tissue damage will not take place over such a short time span. Among the survivors of a dose producing 40% mortality, dangerously high levels of COHb were observed. Furthermore, the mean percent COHb of mice that died from exposure was significantly higher.

As with human subjects, our mice demonstrated tissue damage from smoke. This pathological response is likely a result of the particles in the smoke and noxious chemicals, many of which adhered to the smoke particles, as well as the inflammatory response to these agents. Large quantities of wood, cotton, and polyurethane are commonly found in homes. The initial studies in FVB/n mice showed more lung damage after wood smoke than either cotton or polyurethane smoke. To focus the study, we developed a murine model of wood smoke inhalation in CD-1 mice. In both CD-1 and FVB/n mice, wood smoke caused cell death (cytoplasmic vacuolization, cytoplasmic blebbing, sloughing), infiltration of immune cells (primarily lymphocytes and macrophages), and some peracute hemorrhage and capillary congestion as well as some alveolar hyperplasia and type II cell hypertrophy. The upper airways also demonstrated cell death. Any differences observed between 7 and 9 mg/g exposures are thought...
to be a result of variability in the murine response and not a result of dose.

Cytoplasmic vacuolization and cytoplasmic blebbing demonstrated cell death. Vacuolization was often accompanied by pychnotic nuclei, showing the progression of cell death. The bronchiolar sloughing was yet another indication of cell death and the severity of the lung damage. Sloughed cells, together with mucus, may form respiratory casts that produce airway obstruction and respiratory distress. Cell death may be a result of several factors including tissue hypoxia (2, 20) or damage by chemicals that have adhered to the smoke particles, reactive oxygen species (3) or proteases, cytokines, and other inflammatory mediators. Wood smoke contains large amounts of hydrogen cyanide and aldehydes (19, 29) such as acrolein. Aldehydes are irritants of mucous membranes, inducing denaturation of proteins, causing cellular death, pulmonary edema, and death (33).

The prominent increase in the BAL fluid cell counts after smoke exposure is consistent with that observed in humans as well as in other animal smoke models (6, 15). Furthermore, because smoke inhalation damage is mediated in part through a significant immune response, an increase in immune cells is not only expected but is consistent with the pathology findings.

TNF-α is a potent proinflammatory cytokine associated with lung injury. TNF-α levels have been demonstrated to be elevated in the BAL fluid of acute respiratory distress syndrome patients (4), and acute respiratory distress syndrome is a major cause of mortality in fire victims. Furthermore, direct administration of TNF induces lung endothelial injury, perivascular edema, and extravasation (13). Therefore, the role of TNF-α in the pathways that lead to smoke damage in this murine model was evaluated. Because TNF-α levels in the lavage fluid of mice exposed to smoke were not elevated compared with those of the nonexposed mice, it was concluded that TNF-α release was not stimulated by smoke. These values were compared with a positive control in which cells were stimulated with LPS, yielding a high TNF-α level; lavage fluid macrophages are therefore competent to release TNF-α and increased TNF-α can be detected by the ELISA assay used. However, because smoke exposure did not stimulate increases in TNF-α levels, TNF-α does not appear to mediate the damage in this murine model of smoke inhalation injury. These findings are consistent with a study (14) in sheep exposed to smoke, which demonstrated no detectable increase in TNF-α.
levels after smoke exposure, although endotoxin alone stimulated an increase in TNF-α levels. Alternatively, TNF-α levels may be detectable much earlier in the postexposure period than was measured in this model.

To evaluate the effects of smoke inhalation injury on lung function in mice, static lung compliance was measured. Smoke has been demonstrated to reduce lung compliance in several studies in sheep and dogs (10, 25, 26, 28). In contrast, this murine model of smoke inhalation injury demonstrated no significant changes in static lung compliance. The edema observed did not progress into the intra-alveolar regions to form hyaline membranes as observed in hyperoxic lung injury (22). During hyperoxic lung injury, the serum proteins in the intra-alveolar edema fluid inactivate pulmonary surfactant, resulting in the observed reductions in lung compliance (22). Any direct effects of smoke particles on pulmonary surfactant are also not sufficient to cause significant reductions in lung compliance.

To summarize, this murine model of lung injury reflects the injury and mortality observed in human patients and is therefore useful both for elucidating the mechanisms of smoke inhalation lung injury and for the development of treatment strategies. Due to the availability of inbred strains, the ability to target specific genes and genetically target specific cell types (12, 31), the mouse may prove to be an excellent experimental model in which to evaluate and develop treatment strategies for lung injury. In contrast to the methods of smoke exposure used in numerous other animal models (5, 21, 25), this murine smoke chamber allows for control of the environment around the animal to simulate conditions in structural fires. As with hyperoxic lung damage, much of the lung damage from smoke inhalation is the result of a vigorous inflammatory response. Therefore, future studies will include an extensive examination of the protective effects of the immunosuppressive agent cyclosporin A administered after smoke exposure. Cyclosporin A may inhibit or reduce the pathology observed in smoke-treated lungs similar to its action in hyperoxia-treated lungs (22).

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REFERENCES


