Smoke inhalation—induced alveolar lung injury is inhibited by hyperbaric oxygen

S. R. THOM, I. MENDIGUREN, and D. FISHER

Institute for Environmental Medicine and Department of Emergency Medicine, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania

Thom SR, Mendiguren I, Fisher D. Smoke inhalation—induced alveolar lung injury is inhibited by hyperbaric oxygen. Undersea Hyper Med 2001; 28(4):175–179. —Smoke-induced lung injury in rats was assessed in terms of histopathology, gross mortality, neutrophil accumulation and as capillary leak. Administration of hyperbaric oxygen (HBO$_2$), 2.8 atm abs for 45 min, inhibited adhesion of circulating neutrophils subsequent to smoke inhalation. HBO$_2$ reduced pulmonary neutrophil accumulation whether used in a prophylactic manner, 24 h before smoke inhalation, or as treatment immediately after the smoke insult. Emphasis was placed on prophylactic administration of HBO$_2$ to avoid the possibility that beneficial effects may be related to hastened removal of carbon monoxide. Based on all parameters tested, smoke inhalation injury was reduced by prophylactic administration of HBO$_2$. The beneficial effect appears related to inhibition of neutrophil adhesion to the vasculature.

myeloperoxidase, pneumonitis, respiratory distress syndrome, cell adhesion

Smoke inhalation is the leading cause of death in victims of structural fires (1). Over half of all deaths are related to toxic gases, such as carbon monoxide (CO) and cyanide, and the majority of the remaining mortality is due to lung injuries (2,3). Pulmonary pathophysiology is initially localized to the conducting airways and characterized by edema and tracheobronchitis. At this early stage, only patchy parenchymal lung injury is present (4). Among patients who sustain smoke inhalation and survive the acute threat from toxic gases, mortality occurs in only 5–10 % due to early lung injury. If the injury progresses after the initial 18–24 h, however, it is associated with alveolar inflammatory changes that are often fatal. The clinical variable that heightens risk for developing this form of respiratory distress syndrome is a concurrent cutaneous burn. The mortality risk increases dramatically even in patients with a relatively small total body surface area burn, to as much as 60% in some studies (5–9).

Clinical evidence suggests that products of smoke, or those produced by oxidation of airway tissues, activate alveolar macrophages (10–12). Chemoattractants produced by the alveolar macrophages are believed to cause influx of circulating polymorphonuclear leukocytes (PMN). The risk for lethal alveolar inflammation is greatest when the recruited PMN are already stimulated, or "primed", by additional stimuli. Thermal burns can cause activation of complement in the vascular compartment and circulating PMN appear to have been activated, based on chemotaxis studies and the presence of increased numbers of cell-surface receptors (13–15).

Polymorphonuclear leukocytes play a central role in experimental smoke-induced alveolar lung pathology and little injury develops in neutropenic animals (16,17). Methods of impeding adherence between PMN and endothelium are frequently beneficial for limiting inflammatory injuries, and monoclonal adherence-blocking antibodies have been successfully applied in a smoke inhalation model (18,19). Based on prior investigations, we hypothesized that hyperbaric oxygen (HBO$_2$) may be protective against pulmonary injury because it will inhibit PMN adherence to endothelium. Inhibition of PMN β_2 integrins and their ICAM endothelial counter-receptors can be achieved with HBO$_2$ without compromising immune surveillance and antibacterial functions (20–24). HBO$_2$ exposure will also inhibit PMN β_2 integrin-dependent adherence in humans (25).

This study was designed to evaluate the potential for prophylactic HBO$_2$ to abate the progression of alveolar lung injury from smoke. Although this is obviously not the typical clinical scenario, emphasis was placed on exposures performed before the smoke insult occurred in order to avoid difficulties with interpretation of results. If used after the smoke insult, some beneficial actions of HBO$_2$ might be related to hastened removal of CO from hemoproteins.

Critiques of animal studies point out that their relevance may be challenged because of variations in the

toxic components of smoke and by the progression of pulmonary injury due to inflammation (2). Indeed, because clinical risk is greatest with the combination of injury to the local airways coupled with a cutaneous burn, few relevant models exist. Incorporating both variables in a humane model requires concomitant anesthesia, which may add further hemodynamic compromise (26). Moreover, thermal injury can by itself cause diffuse capillary leakage (27). Use of smoke and thermal burns, plus anesthetics, has been performed in one sheep and one mouse model (26,28–30). To avoid the confounding variable posed by anesthetics, we chose a laboratory model using spontaneously breathing, unanesthetized rats. Local lung injury in this model can be varied by using different fuels to produce smoke, and circulating PMN are remotely activated by inducing peritonitis with sterile glycogen (17).

METHODS

Animals and reagents: Male Fischer rats (weighing 200–250 g) certified pathogen-free (Charles River Laboratories, Wilmington, DE) were fed a standard diet and water ad libitum. Scrubbed nylon (type 200L) was produced by DuPont Biotechnology Systems Division (Boston, MA). All other reagents were purchased from Sigma Chemical Co., St. Louis, MO.

Animal procedures: The smoke inhalation model and all treatments given to the animals were approved by the Institutional Animal Care and Use Committee. Rats received an intraperitoneal injection of sterile oyster shell glycogen, 6 ml of 1% wt/vol, 4 h before smoke exposure. They were exposed, head only, for 30 min to smoke generated from the non-flaming combustion of polyvinyl chloride combined with Douglas fir wood, as described previously (17). Typically, four to six rats were exposed at a time. Untreated control rats were included with all repeated experiments to assure comparability among studies, which lead to slightly unequal total numbers of rats in the different treatment groups. Except were indicated, parameters were measured 24 h after exposure to smoke. Lung [^{125}I]albumin retention, myeloperoxidase (MPO) activity, and histologic examinations were per-

formed as described in detail previously (17). In brief, rats were anesthetized [0.25 mg $\cdot$ kg^{-1} ketamine and 5 mg $\cdot$ kg^{-1} xylazine i.p.], lungs exposed, and the pulmonary artery cannulated with a 22-gauge catheter. The left atrium and ventricle were opened widely to allow free drainage of blood. For the [^{125}I] albumin retention and MPO assays, lungs were perfused with phosphate buffered saline containing 4% Ficoll and homogenized for analysis. A 1-ml aliquot of blood was also assayed to allow correction for residual blood in the lung homogenate. Residual hemoglobin in the lung homogenate was measured using the dithionite assay and the lung homogenate value corrected based on results obtained with the 1-ml blood aliquot (17). Contaminating blood averaged less than 20 ul. For histologic analysis, the chest and abdominal cavity were opened to allow the lungs to collapse. The trachea was cannulated with a tube leading from a reservoir of fixative [4% (wt/vol) formalin in 300 mOsM phosphate buffer] located 20 cm above the level of the sternum and perfused under gravity to fix the lungs in an expanded state (17). Exposure to HBO$_2$ was carried out at 2.8 atm abs for 45 min following published procedures (22). PMN adherence was evaluated by passing heparinized whole blood through columns of scrubbed nylon fibers as described in previous publications (22).

Statistical analysis: Data are reported as mean $\pm$ SE, with significant differences determined by one-way analysis of variance using the Bonferroni/Dunn post hoc test.

RESULTS

Clinical progression of injuries: Rats were exposed to smoke from Douglas fir wood plus polyvinyl chloride plastic, a combination that was reported to cause severe lung injury (17). Four hours before the smoke exposure, they received an intraperitoneal injection with sterile glycogen. Lung pathology was evaluated in rats killed 24 h following smoke inhalation. Table 1 demonstrates that all rats, whether they received HBO$_2$ or not, developed tracheobronchitis, which is consistent with a local response to smoke products. However, significantly fewer rats who received prophylactic HBO$_2$ developed pneumonitis.

Table 1:Number of Rats Demonstrating Various Types of Pulmonary Histopathology 24 h After Exposure to Smoke[a]

Group	Total No. Examined	Tracheo-bronchitis	Perivascular Bronchial Edema	Alveolar Infiltrates	Pulmonary (Alveolar) Edema
Smoke	$n = 8$	8	4	7	7
HBO$_2$ + smoke	$n = 7$	7	2	1*	1*

[a]Observations were made by investigator masked to exposure group.   *$P < 0.05$. No pathologic changes were observed in five rats examined after exposure to only HBO$_2$.

Table 2: Mortality From Smoke Inhalation

	Acute Death, < 4 h	Death at 18–48 h
Smoke, no treatment, $n = 29$	3, COHb 84 ± 1%	6
HBO$_2$ then smoke, $n = 20$	1, COHb 80%	3*

*$P < 0.05$.

This model was developed with the aim to minimize spontaneous mortality, as that would compromise the reliability of pathologic evaluations. Nonetheless, a few animals died spontaneously. Acute mortality was defined as death less than 4 h after smoke exposure. All rats dying in the acute time interval had COHb levels of 80%, or more. No other rats died until 18 or more hours later. We observed that late deaths occurred in fewer rats who had received prophylactic HBO$_2$, as shown in Table 2.

Lung myeloperoxidase activity: Neutrophil localization within lungs 24 h after the smoke exposure was quantified by measuring activity of MPO in tissue homogenates. As shown in Fig. 1, there was a significant elevation in MPO activity due to smoke, and HBO$_2$ reduced MPO content whether administered 24 hours before the smoke exposure or immediately after smoke inhalation.

Circulating neutrophil adherence: Adherence of circulating PMN was measured by filtering blood through nylon columns. Tests were conducted after rats were exposed to smoke, and at comparable times in those

FIG. 2—PMN adherence to nylon columns. Heparinized blood was obtained from rats exposed only to air (control), 4.5 h after intraperitoneal injection with glycogen, immediately after the 30-min exposure to smoke, after intraperitoneal glycogen injection followed by a 4-h delay and then a 30-min exposure to smoke, or HBO$_2$ exposure the day before intraperitoneal glycogen injection followed by a 4-h delay and then a 30-min exposure to smoke. Data represent % PMN that adhered to nylon expressed as mean ± SE (n = number of rats studied). *$P < 0.05$ vs. control.

rats exposed to individual components of the model. Therefore, rats were studied immediately after the 30-min exposure to smoke, 4.5 h after intraperitoneal injection with glycogen, after intraperitoneal glycogen injection followed by a 4-h delay and then a 30-min exposure to smoke, or HBO$_2$ exposure the day before intraperitoneal glycogen injection followed by a 4-h delay and then 30-min exposure to smoke. PMN adherence was not significantly different except among rats receiving HBO$_2$ before smoke inhalation (Fig. 2). These data are consistent with prior publications (21,22). They indicate that adherence function of circulating neutrophils was inhibited by HBO$_2$ and that the smoke plus glycogen insult did not modify this response.

Capillary permeability: Measurements of [^{125}I]albumin retention were made on lungs from rats 24 h after the smoke insult. Results (Fig. 3) demonstrated a capillary leak in lungs from rats exposed to the smoke insult and significantly less leakage of albumin into lung tissues if rats were treated with HBO$_2$ before smoke inhalation.
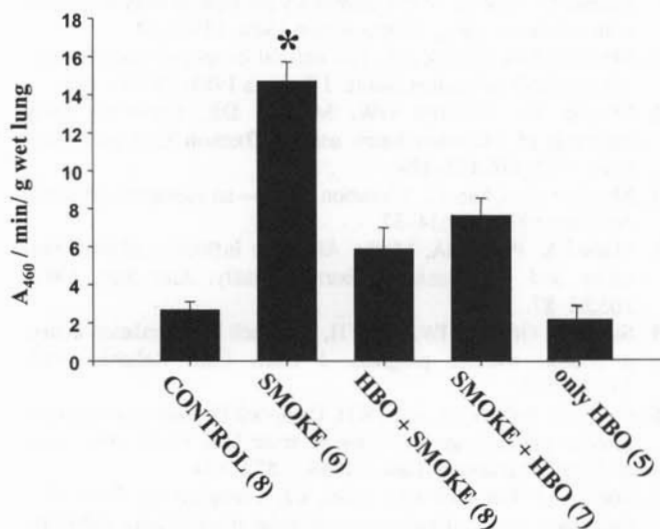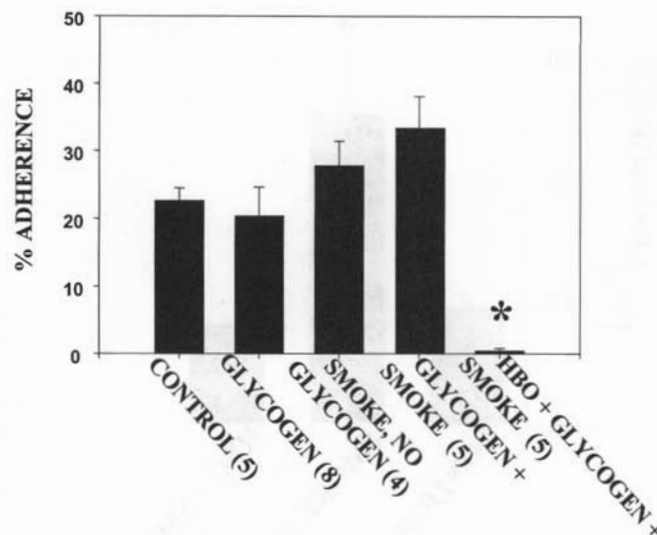

FIG. 1—Lung PMN infiltration evaluated by MPO activity. MPO activity was measured 24 h after smoke exposure. Results are expressed as change in absorbance of preparation at 460 nm · min^{-1} per gram wet weight of lung (mean ± SE, *$P < 0.05$ compared with control).

DISCUSSION

Smoke-induced lung injury was assessed in terms of histopathology, gross mortality, PMN accumulation, and as capillary leak. Administration of HBO$_2$ reduced PMN accumulation whether used in a prophylactic manner or as treatment after the smoke insult. Based on all param-
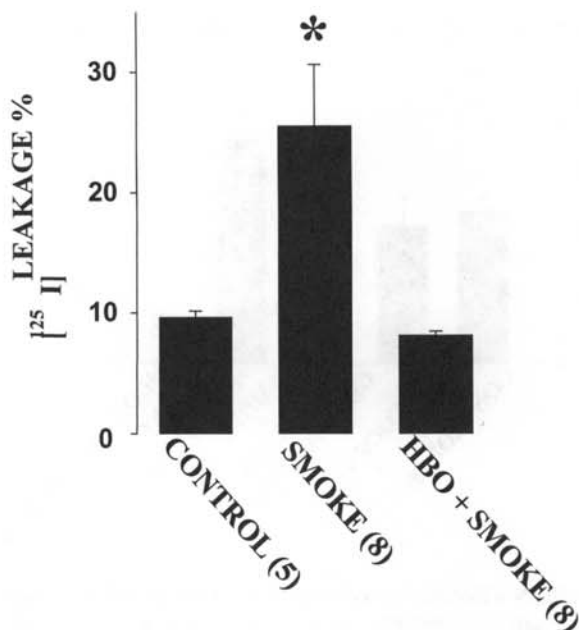
FIG. 3—[^{125}I]Albumin permeability of lung 24 h after exposure to smoke. Data are expressed as the percentage of counts present in lung homogenate vs. counts in blood from same animal (mean ± SE, *$P <$ 0.05 vs. control, n = number of rats studied).

eters tested, smoke inhalation injury was reduced by prophylactic administration of HBO$_2$. In prior investigations, protection against lung injury was shown with neutropenia and with agents that inhibited elaboration of reactive species (31). Data presented in this paper suggest that the beneficial effect of HBO$_2$ is related to its ability to inhibit PMN adhesion function. Impaired adhesion of circulating PMN subsequent to smoke inhalation was shown in rats treated with HBO$_2$.

Investigating the pathophysiology and treatment of smoke inhalation is confounded by the variability of the clinical picture. Smoke itself has variable composition and host factors add to variability of responses (2). The focus of this paper was on mechanisms responsible for development of alveolar injury, and limitations in the applicability of conclusions are obviously dependent of the model used. Ours is a model initially developed at the National Institute of Standards and Technology, and the smoke generated in this model system has been shown to closely approximate that which is found in large-scale structural fires (32). The model also allows for avoidance of anesthesia, which is required with large animal studies requiring surgical instrumentation and models where thermal burns are inflicted. Glycogen peritonitis, an obvious artificial stimulus, was used in place of burns to precipitate an inflammatory response which does not itself alter the lungs but will modify responsiveness of circulating PMN (33).

Practical application of the results in this paper are

supported by anecdotal reports of reduced lung injury among patients who received HBO$_2$ for thermal burns after house fires (34). HBO$_2$ has been shown to inhibit rat PMN β$_2$ integrin adhesion function, as measured by nylon filtration and other assays, and the effect persists for at least 24 h (22). In humans, the effect of HBO$_2$ persists for less than 12 h (25). The reason for the difference in duration of the effect is unknown as the half-life for circulating PMN is similar; 6.6 h in humans and approximately 5.7 h in rats (35, 36).

Our results suggest that there is merit to further investigations into the potential benefit of HBO$_2$ for lung injury from smoke inhalation. Workers should remain mindful, however, of the limitations in our study. The fidelity of any animal model to replicate clinical injury is always a question. Moreover, the time course for development of lung injury, and the window of opportunity for successful therapeutic interventions, may vary based on environmental and host factors.

REFERENCES

1. Demling RH. Smoke inhalation injury. New Horiz 1993; 1:422–434.
2. Schulz III, JT, Ryan CM. The frustrating problem of smoke inhalation injury. Crit Care Med 2000; 28:1677–1678.
3. Thompson PB, Herndon DN, Traber DL, Abston S. Effect on mortality of inhalation injury. J Trauma 1986; 26:163–165.
4. Herndon DN, Traber LD, Linares H, Flynn JD, Niehaus G, Kramer G. Etiology of the pulmonary pathophysiology associated with inhalation injury. Resuscitation 1988; 14:43–49.
5. Marshall WG, Dimick AR. The natural history of major burns with multiple subsystem failure. J Trauma 1983; 23:102–105.
6. Moylan JA, Wilmore DW, Mouton DE, Pruitt BA. Early diagnosis of inhalation injury using 133xenon lung scan. Ann Surg 1972; 176:477–484.
7. Moylan JA, Chan C. Inhalation injury—an increasing problem. Ann Surg1978; 188:34–37.
8. Shirani K, Pruitt BA, Mason AD. The influence of inhalation injury and pneumonia on burn mortality. Ann Surg 1987; 205:82–87.
9. Sobel JB, Goldfard IW, Slater H, Hammell EJ. Inhalation injury: a decade without progress. J Burn Care Rehabil 1992; 13:573–575.
10. Clark CJ, Pollock AJ, Reid WH, Campbell D. Role of pulmonary alveolar macrophage activation in acute lung injury after burns and smoke inhalation. Lancet 1988; 2:872–874.
11. Pollok AJ, Gemmell CG, Clark CJ, Campbell D, Reid WH. Functional status of pulmonary alveolar macrophages following exposure to toxic gases. Br J Anaesth 1987; 59:943.
12. Riyami BMS, Tree R, Kinsella J, Clark CJ, Reid WH, Campbell D. Changes in alveolar macrophage, monocyte, and neutrophil cell profiles after smoke inhalation injury. J Clin Pathol 1990: 43:43–45.
13. Moore FD, Davis C, RodrickM, Mannick JA, Fearon DT. Neutrophil activation in thermal injury as assessed by increased

expression of complement receptors. N Engl J Med 1986; 314:948–953.

14. Nelson RD, Hasslen SR, Ahrenholz DH, Haus E, Solem LD. Influence of minor thermal injury on expression of complement receptor CR3 on human neutrophils. Am J Pathol 1986; 125: 563–570.

15. Solomkin JS. Neutrophil disorders in burn injury: complement, cytokines, and organ injury. J Trauma 1990; 30:S80–S85.

16. Basadre JO, Sugi K, Traber DL, Traber LD, Niehaus GD, Herndon DN. The effect of leukocyte depletion on smoke inhalation injury in sheep. Surgery 1988; 104:208–215.

17. Thom SR, Mendiguren I, VanWinkle T, Fisher D, Fisher AB. Smoke inhalation with a concurrent systemic stress results in lung alveolar injury. Am J Respir Crit Care Med 1994; 149:220–226.

18. Sakurai H, Schmalstieg FC, Traber LD, Hawkins HK, Traber DL. Role of L-selectin in physiological manifestations after burn and smoke inhalation injury in sheep. J Appl Physiol 1999; 86:1151–1159.

19. Virkhaus RB, Lucchesi R, Simpson PJ, Shebuski RJ. The role of adhesion molecules in cardiovascular pharmacology: meeting review. J Pharmacol Exp Ther 1995; 273:569–575.

20. Buras JA, Stahl GL, Svoboda KKH, Reenstra WR. Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. Am J Physiol 2000; 278:C292–C302.

21. Chen Q, Banick PD, Thom SR. Functional inhibition of rat polymorphonuclear leukocyte β_2 integrins by hyperbaric oxygen is associated with impaired cGMP synthesis. J Pharmacol Exp Ther 1996; 276:929–933.

22. Thom SR. Functional inhibition of leukocyte β_2 integrins by hyperbaric oxygen in carbon monoxide-mediated brain injury in rats. Toxicol Appl Pharmacol 1993; 123:248–256.

23. Tjarnstrom J, Wikstrom T, Bagge U, Risberg B, Braide M. Effects of hyperbaric oxygen treatment on neutrophil activation and pulmonary sequestration in intestinal ischemia-reperfusion in rats. Eur Surg Res 1999; 31:147–154.

24. Zamboni WA, Roth AC, Russell RC, Smoot EC. The effect of hyperbaric oxygen on reperfusion of ischemic axial skin flaps: a laser doppler analysis. Ann Plastic.Surg 1992; 28:339–341.

25. Thom SR, Mendiguren I, Hardy KR, et al. Inhibition of human neutrophil B2-integrin dependent adherence by hyperbaric O_2.

Am J.Physiol 1997; 272:C770–C777.

26. Matsumoto N, Noda H, Nakazawa H, Traber LD, Herndon DN, Traber DL. The sequence of injury determines the degree of lung damage in both inhalation and thermal injuries. Shock 1994; 1:166–170.

27. Demling RH, Will JA, Belzer FO. Effect of major thermal injury on the pulmonary microcirculation. Surgery 1978; 83:746–751.

28. Alpard SK, Zwischenberger JB, Tao W, Deyo DJ, Traber DL, Bidani A. New clinically relevant sheep model of severe respiratory failure secondary to combined smoke inhalation/cutaneous flame burn injury. Crit Care Med 2000; 28:1469–1476.

29. Soejima K, Schmalsteig FC, Sakurai H, Traber LD, Traber DL. Pathophysiological analysis of combined burn and smoke inhalation injuries in sheep. Am J Physiol 2001; 280: L1233–L1241.

30. Zawacki BE, Jung RC, Joyce J, Rincon E. Smoke, burns, and the natural history of inhalation injury in fire victims. Ann Surg 1977; 185:100–110.

31. Ischiropoulos H, Mendiguren I, Fisher D, Fisher AB, Thom SR. Role of neutrophils and nitric oxide in lung alveolar injury from smoke inhalation. Am J Respir Crit Care Med 1994; 15:337–341.

32. Levin BS, Fowell AJ, Birky MM, Paabo M, Stolte A, Malek D. Further development of a test method for the assessment of the acute inhalation toxicity of combustion products. Washington, DC: National Bureau of Standards 1982. (publ no 82-2532).

33. Freyer DR, Morganroth ML, Todd RF. Surface Mol (CD11b/CD18) glycoprotein is up-modulated by neutrophils recruited to sites of inflammation in vivo. Inflammation 1989; 13:495–505.

34. Ray CS, Green B, Cianci P. Hyperbaric oxygen therapy in burn patients with adult respiratory distress syndrome. Undersea Biomed Res 1989; 16(suppl.):81.

35. Athens JW, Haab OP, Raab SO, et al. Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. J Clin Invest 1961; 40:989–995.

36. Gerecke D, Schultze B, Maurer W. Kinetics of neutrophilic granulocytes in the blood of rats. Cell Tissue Kinet 1973; 6:369–378.