Carbon Monoxide Effect on Alveolar Epithelial Permeability*

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A carbon monoxide (CO)-intoxicated patient developed increased permeability-type pulmonary edema demonstrated by a normal capillary wedge pressure and production of protein-rich edema fluid. To investigate the effect of CO on alveolar-epithelial permeability, a radioactive labelled isotope, ⁵¹Cr-EDTA (MW 377), was instilled into the airways of rabbits. Subsequent egress of the marker from the lungs into arterial blood was determined in serial arterial blood samples. The ⁵¹Cr-EDTA counts increased significantly within 15 minutes in the CO-exposed animals, compared with the control animals, while dynamic lung compliance fell, airways resistance rose, and arterial blood pressure decreased. Ultrastructural study of the lungs of CO-exposed animals revealed epithelial and endothelial cell swelling, interstitial edema, and alveolar type II cells depleted of lamellar bodies. These findings support the possibility that carbon monoxide intoxication is associated with increased alveolar-epithelial permeability.

Carbon monoxide (CO) poisoning is a common cause of accidental and suicidal death.¹ The toxic effect of CO exposure has been commonly attributed to the reduction of oxygen transport to tissues because of loss of oxygen combining sites on hemoglobin molecules and shift of the hemoglobin-oxygen dissociation curve to the left. However, little attention has been paid to the effect of CO on cell metabolism. It has been suggested that CO exerts a more general effect on oxidative metabolism by depressing cytochrome systems throughout the body;² accordingly, lung damage might result from interference with normal oxidative pathways in the alveolar epithelium and capillary endothelium.

Clinical reports have indicated that CO intoxication is accompanied by abnormal results of physical examination of the chest and abnormal gas exchange.³ Whorton⁴ noted a mean alveolar-arterial oxygen difference of 55 mm Hg in 14 patients with carboxyhemoglobin levels ≥ 19 percent, while Sones et al⁵ found roentgenographic evidence of interstitial and alveolar edema in 11 of 18 patients with acute CO intoxication. In more than one half of 567 patients who died of CO intoxication, Finck⁶ found pulmonary edema and hemorrhage at post mortem pathologic examination. Despite these findings, the relationship between CO poisoning and pulmonary edema has not been clearly delineated.

A recent case in which permeability-type pulmonary edema followed severe CO intoxication led us to investigate the effect of CO on alveolar-epithelial permeability. The clinical data for this patient and the results of studies demonstrating increased alveolar-epithelial permeability in a rabbit model of CO intoxication are reported herein.

**CASE REPORT**

A 27-year-old previously-healthy woman was admitted to San Francisco General Hospital Medical Center on Feb 14, 1978, after several hours of exposure to auto exhaust. The patient was comatose with a respiratory rate of 28 breaths per minute, heart rate of 140 beats per minute, temperature of 38.8°C, and blood pressure of 100/50 mm Hg. Cardiovascular and pulmonary examinations showed no abnormalities; severe neurologic damage was evidenced by decorticate rigidity. Laboratory investigation revealed a hemoglobin level of 14.5 g/100 ml, a white blood cell count of 23,500/µm, and a platelet count of 300,000/µm. Results of analysis of arterial blood obtained while the patient breathed 100 percent oxygen through a nonrebreathing mask were pH 7.41, Pco₂ 22 mm Hg and Po₂ 471 mm Hg. Despite breathing the increased oxygen for at least 30 minutes, a measured carboxyhemoglobin level was 20 percent. The anteroposterior chest roentgenogram on admission showed no abnormalities. When tachypnea and blood-stained airway foam developed 13 hours after admission, a second roentgenogram was taken and revealed bilateral alveolar and interstitial infiltrates (Fig 1). Because of severe hypoxemia (PaO₂ 50, FIO₂ 0.7), the patient was intubated and placed on mechanical ventilation.
The ratio of protein concentrations in pulmonary edema fluid to plasma protein on the first hospital day was 1.0, indicating a marked increase in permeability. Although the pulmonary capillary wedge pressure consistently remained less than 10 mm Hg, respiratory dysfunction progressed over the next four days with severe hypoxemia (PaO₂, 50 to 70 mm Hg with an FIO₂ between 0.5 and 1.0 and PEEP between 5 and 10 mm Hg) and reduced “effective” thoracic compliance (tidal volume/plateau pressure) (20 to 30 ml/cm H₂O). On the fifth hospital day, the patient died. Post-mortem examination revealed marked intra-alveolar hemorrhage and pulmonary edema with hyaline membrane formation. There were interstitial mononuclear infiltrates scattered throughout the myocardium and occasional small perivascular hemorrhages in the brain. Electron photomicrographs of the lung showed severe epithelial cell damage, alveolar type II cell multiplication, and hyaline membrane formation.

METHODS

The effect of CO on alveolar epithelial permeability was studied in nine New Zealand white rabbits (weight 3.4 ± 1.4 kg). An intramuscular dose of ketamine (60 mg/kg) was administered preoperatively followed by incremental doses of pentobarbital sodium (30 mg/kg) via an ear vein catheter. A tracheostomy was performed and the renal pedicles were exteriorized via a small midline incision, and ligated. Samples of arterial blood were obtained from a femoral artery catheter by heparinized syringes. The animals were paralyzed with succinylcholine chloride (6 mg/kg initially and then 3 mg/kg every 30 minutes thereafter), administered intravenously, and ventilated with a Harvard respirator at a tidal volume of 10 ml/kg. Tracheal air flow was measured with a heated Flesch pneumotachograph, and the volume was derived by electronic integration. Tracheal air flow, tidal volume and airway and arterial pressures were displayed on a Beckman pen recorder. Dynamic compliance and airways resistance were determined from simultaneous measurements of tidal flow, volume, and transpulmonary pressure.

An index of permeability across the alveolar epithelium was derived by a method described previously. Briefly, we followed the rate of passage of an isotopically labelled tracer from alveoli to blood.

A freely diffusible lipophilic tracer, 125I-antipyrine, which passes from alveoli to blood with a half time (T½) of three minutes regardless of permeability, was used to reference the amount of 51Cr-EDTA reaching the alveoli after tracheal instillation. The 51Cr-EDTA, a poorly diffusible hydrophilic tracer, leaves alveoli very slowly (T½ ~ 200 minutes) under normal conditions. When permeability increases, the rate of 51Cr-EDTA egress increases dramatically and can be measured in arterial blood.

A mixture containing the two isotopes (50 μCi each) in 1 ml of 0.9N saline followed by 1 ml of saline flush was injected through the tracheal cannula. Serial 1.2-ml samples of arterial blood were taken every 15 minutes during a 90-minute period before and during a 45-minute period after exposure to CO in five rabbits and in four control rabbits who were not exposed to carbon monoxide. The relative activities of the two tracers were measured using conventional gamma ray-counting equipment with standard corrections for background and crossover between the two isotopes. Alveolar epithelial permeability was assessed by comparing experimental and control groups with respect to 51Cr-EDTA passage into arterial blood.

Following the 90-minute baseline period during which all rabbits were ventilated with air, five rabbits were ventilated with 0.8 percent CO for 45 minutes while four animals continued to breathe air for control measurements. Dynamic compliance, airways resistance, blood pressure, and heart rate were recorded every 15 minutes during the duration of the experiment. Arterial blood gas levels and carboxyhemoglobin levels were analyzed just before, and 15 and 30 minutes after, CO exposure and in the control rabbits.

At the end of the study, the animals were killed by

Figure 1. Bilateral interstitial and alveolar infiltrates seen. Air in mediastinum and subcutaneous tissue is evident. Tube was introduced on right side.

Figure 2. Time course of 51Cr-EDTA counts/minute expressed as a percent of the 90-minute value (immediately before CO exposure) for control and CO-exposed animals. The 51Cr-EDTA counts in arterial blood were significantly greater and increased at a faster rate in animals exposed to CO than in animals breathing room air. There was no significant difference in appearance of 51Cr-EDTA in control and experimental animals during 90-minute baseline period.

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intravenous administration of barbiturate. The lungs were removed for ultrastructural examination of tissue. Results in the experimental and control groups were compared using the Student t-test for unrelated measures.

RESULTS

During the 90-minute baseline period, the $^{51}$Cr-EDTA counts increased progressively and similarly in arterial blood in both groups of animals. Within 15 minutes of beginning CO exposure, the $^{51}$Cr-EDTA counts increased significantly in these rabbits compared with the counts in the control animals ($P < 0.01$) (Fig 2). The increase in $^{51}$Cr-EDTA passage from alveoli to arterial blood in rabbits breathing CO persisted and increased throughout the subsequent 30 minutes of the experiment, ($P < 0.01$) indicating a persistent increase in alveolar epithelial permeability. The mean carboxyhemoglobin level for the four animals exposed to CO was 63 ± 4 percent (mean ± 1 SEM).

Concurrent with the rise in alveolar-epithelial permeability was a fall in dynamic lung compliance and a rise in airways resistance (Fig 3). These differences were significant ($P < 0.05$) at 15 and 30 minutes after CO exposure, respectively. Mean blood pressure fell progressively to 62 percent of the baseline value within 45 minutes after CO exposure ($P < 0.05$) (Fig 3). The heart rate, although slightly elevated, was not significantly different from that of the control rabbits or baseline values (Fig 3).

Results of analyses of arterial blood gases are shown in Figure 4. Arterial pH fell progressively during the carbon monoxide exposure ($P < 0.05$). The alveolar arterial $P(A-a)O_2$ difference showed a tendency to increase, but the change was not statistically significant.

Although morphometric examination was not performed, transmission electron photomicrographic examination of the lungs showed widespread areas of both endothelial and epithelial swelling (Fig 5) and detachment of the endothelium from the basement membrane (Fig 6). There was marked interstitial edema. Mitochondria were severely distinguished and alveolar type II cells were depleted of lamellar bodies. These changes were not present in control animals.

DISCUSSION

In the patient reported herein, permeability-type pulmonary edema clearly developed after prolonged exposure to auto exhaust, established by leakage of protein-rich fluid into alveoli and persistently normal pulmonary capillary wedge pressures. Although the bulk of auto exhaust (87 percent) is CO, we cannot be certain that small quantities of nitrogen dioxide and sulfur dioxide, which are also present, did not contribute to the alteration in alveolar-epithelial membrane permeability. Our animal data, however, indicate that CO alone significantly increases alveolar-epithelial membrane permeability and thereby enables increased filtration of fluid into alveoli. In the rabbits, permeability was altered within 15 minutes of exposure to high concentrations of CO and increased progressively during the subsequent 30 minutes of the experiment. Although overt signs of pulmonary edema such as frothing did not occur, altered alveolar-epithelial permeability...
breathing 100 percent oxygen for at least 30 minutes; thus the T% of carboxyhemoglobin's disappearance was reduced to approximately 40 minutes. We exposed our animals to a level of CO that we believe simulated the peak concentration in our patient in whom permeability pulmonary edema occurred.

Direct comparison of the pathologic findings in our patient and rabbit model of CO intoxication is difficult because of different durations of exposure to CO, and therapeutic interventions. Despite these limitations, damage to the alveolar-epithelium was common to both. In our patient, there were many areas of denuded epithelium, hyaline membranes, and regenerating type II cells indicative of the more prolonged time course following alveolar injury. In the rabbits, with a brief but concentrated exposure to CO, ultrastructural examination revealed alveolar

was accompanied by a reduction in dynamic compliance, an increase in airways resistance and electron micrographic evidence of interstitial pulmonary edema.

The measured carboxyhemoglobin concentration in our patient (50 percent) was considerably less than her estimated peak level because the blood sample was obtained after the patient had been removed from the site of exposure and had been

Figure 5. In rabbit, electron micrograph demonstrates marked swelling of alveolar type I cell is shown. AS indicates alveolar space; BM, basement membrane; EPI, epithelium; and END, endothelium. Line is 2μm (original magnification × 13,600, PTAH stain).

Figure 6. In rabbit, electron micrograph demonstrates separation of endothelium from basement membrane is shown. AS, indicates alveolar space; BM, basement membrane; END, endothelium; RBC, red blood cell; and IS, interstitial space. Line is 2μm (original magnification × 11,000, PTAH stain).
epithelial and endothelial cell swelling, separation of the endothelium from the basement membrane, and disappearance of lamellar bodies from alveolar type II cells. Because we did not study either the ultrastructural sequelae of prolonged CO exposure or the result if recovery over several days had been allowed, we cannot be certain that the early pathologic changes seen in the rabbits would have progressed to the pathologic abnormalities present in our patient post mortem.

The mechanism of the observed increase in lung permeability has not been definitively answered by this study. A number of factors either singly or in part may contribute to the permeability alteration. Since pulmonary capillary wedge pressure was not measured in the rabbits, we cannot be certain of the contribution of heart failure to the experimental model. However, studies by Egan and coworkers are helpful in suggesting that the observed increase in 51Cr-EDTA egress from alveoli to arterial blood following CO intoxication is specific for a permeability alteration. They showed that in dogs with acute hemodynamic pulmonary edema, 125I-albumin passed readily from the capillary bed into air spaces, but that 125I-albumin did not pass from air spaces into blood. This was in marked contrast to the findings in dogs with alloxan-induced increased permeability pulmonary edema where bidirectional transfer was evident. Thus, transfer of radioactive labels from air spaces to blood as in our model of CO intoxication appear unique for increased permeability pulmonary edema.

Significant systemic hypotension occurred in the rabbits after approximately 30 minutes of CO exposure. Since a significant permeability change was evident after only 15 minutes of CO exposure, it is unlikely that hypotension alone can be implicated as a causative factor. Moreover, Demling and co-workers, in elegant studies on both anesthetized and awake sheep, have shown that hemorrhagic shock has no effect on pulmonary vascular protein permeability.

The rabbits became severely acidotic after 30 minutes of CO exposure. Because severe acidosis interferes with cellular function, a permeability alteration might be explained by this factor. However, in a separate study of rabbits with extensive surface burns and profound metabolic acidosis, we could detect no change in alveolar epithelial permeability.

In the patient described herein, severe neurologic dysfunction was present as evidenced by decorticate rigidity. Such severe neurologic damage may be associated with pulmonary edema. The mechanism for neurogenic pulmonary edema has been postulated to involve a centrally mediated, massive, sympathetic discharge which would produce a transient but intense vasoconstriction with a resultant shift of blood from the systemic circulation to the pulmonary circulation. The resultant increased pulmonary capillary pressure leads to hydrostatic pulmonary edema. The striking pulmonary hypertension and hypervolemia injure pulmonary blood vessels leading to altered capillary permeability. When the altered hemodynamics return to normal, increased capillary permeability persists. These changes can be prevented by the prior administration of adrenergic blockers. We have observed similar changes in permeability in rabbits after the intravenous injection of large doses of adrenalin. Since we did not measure pulmonary capillary wedge pressure in the rabbits, the sequence of events described above could have occurred. However, we found that the pulmonary edema encountered with adrenalin was associated with extensive intrapulmonary hemorrhage, whereas there was little evidence of hemorrhage in the CO-exposed animals.

Carbon monoxide interferes with oxidative reduction reactions involving cytochromes. Mitochondria, the principal site of oxygen utilization and adenosine triphosphate synthesis, are present in all parenchymal lung cells, and the highest concentrations are found in the metabolically active type II cells. Rhodes demonstrated that CO completely inhibits oxygen consumption in vitro in mouse lung slices and the inhibition is reversed when CO is flushed from the system. At carboxyhemoglobin levels below 10 percent in mice, Niden observed fragmentation of lamellar bodies and dilation of the smooth endoplasmic reticulum in alveolar type II cells. Kjeldsen et al showed that endothelial swelling occurs in the myocardium of rabbits chronically exposed to low levels of CO. The prolonged exposure of the alveoli to CO may have interfered sufficiently with enzyme action to cause loss of alveolar epithelial integrity.

Fisher et al, using an experimental system that exposed only one lung of dogs to low levels of CO, found no alterations in diffusing capacities, pressure volume curves, or lung morphology by electron or light microscopy on the CO-exposed side. He suggested that the severe pulmonary damage seen in experimental and clinical CO intoxication is the result of systemic hypoxia related to CO interference with oxygen transport. In our study, it was not possible to separate the direct effects of CO on cellular metabolism from those on oxygen transport. Given the extent and duration of CO intoxication in our animals, it is likely that these factors were addi-
tive in damaging the alveolar epithelium.

In the lung, the alveolar epithelium represents the major barrier to transport of large molecules into the alveolus. Calculations have shown that pores in the capillary endothelial membrane are approximately ten times as large as those in the alveolar epithelial membrane. The anatomic lesions we observed suggest a breakdown in the alveolar epithelium, which in its most severe form may lead to alveolar flooding and the clinical picture of adult respiratory distress. It is tempting to speculate that such a progressive increase in permeability following CO intoxication accounted for the clinical picture in our patient.

In summary, we have described functional and structural changes of the alveolar epithelium that accompany CO intoxication in a human and in an animal model. Increased alveolar epithelial permeability allows increased filtration of fluid into the lung, thereby increasing the risk of alveolar flooding. Fluid restriction to decrease hydrostatic driving pressure with monitoring of pulmonary capillary wedge pressure if necessary may minimize the extent of pulmonary edema in patients intoxicated with CO.

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