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Pulmonary Phototherapy to Treat Carbon Monoxide Poisoning in Rats

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Abstract

Background—Carbon monoxide (CO) poisoning is a common cause of poison-related mortality. CO binds to hemoglobin in the blood to form carboxyhemoglobin (COHb), impairing oxygen delivery to peripheral tissues. Current treatment of CO poisoned patients involves oxygen administration to rapidly remove CO and restore oxygen delivery. Light dissociates CO from COHb with high efficiency. Exposure of murine lungs to visible laser-generated light improved the CO elimination rate *in vivo*. The aims of this study were to apply pulmonary phototherapy to a larger animal model of CO poisoning, to test novel approaches to light delivery, and to examine the effect of chemiluminescence-generated light on the CO elimination rate.

Methods—Anesthetized and mechanically ventilated rats were poisoned with CO and subsequently treated with air or oxygen combined with or without pulmonary phototherapy delivered directly to the lungs of animals at thoracotomy, via intrapleural optical fibers or generated by a chemiluminescent reaction.

Results—Direct pulmonary phototherapy dissociated CO from COHb reducing COHb half-life by 38%. Early treatment with phototherapy in critically CO poisoned rats improved lactate clearance. Light delivered to the lungs of rats via intrapleural optical fibers increased the rate of CO elimination without requiring a thoracotomy, as demonstrated by a 16% reduction in COHb half-life. Light generated in the pleural spaces by a chemiluminescent reaction increased the rate of CO elimination in rats breathing oxygen, reducing the COHb half-life by 12%.

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Conflict of Interest

MGH has filed a patent application on pulmonary phototherapy and CO removal for treating CO poisoning.

Conclusions—Successful application of pulmonary phototherapy in larger animals and humans may represent a significant advance in the treatment of CO poisoned patients.

Keywords

Carbon monoxide; phototherapy; poisoning; carboxyhemoglobin

INTRODUCTION

Each year, acute carbon monoxide (CO) poisoning is responsible for more than 50,000 visits to emergency departments in the United States (1, 2). CO binds to hemoglobin to form carboxyhemoglobin (COHb) with an affinity 200 fold higher than oxygen, impairing oxygen delivery to peripheral tissues (3, 4). CO also binds to other heme-containing proteins such as cytochromes (5, 6) and myoglobin (7, 8), causing direct tissue damage (9 - 11).

Early treatment of CO poisoned patients using normobaric 100% oxygen (NBO) facilitates CO removal and restores oxygen delivery (12, 13). Both hyperbaric oxygen therapy (HBO) (14 - 16) and isocapnic hyperventilation (IHO) (17 - 19) can double the rate of CO elimination as compared to NBO. Increasing ventilation and cardiac output by exercising healthy subjects on a cycle ergometer further increased the rate of CO elimination (20). However, HBO requires patient transportation to centers with specialized equipment and IHO requires a large increase in minute ventilation, which may be uncomfortable in awake patients. Exercising CO poisoned patients with altered mental status or coma is not feasible and administration of drugs that increase cardiac output is dangerous in patients with CO-induced hemodynamic instability and cardiac dysfunction.

Light selectively dissociates CO from hemoglobin, without affecting oxygen binding affinity for hemoglobin (21-22). A wide range of light wavelengths (260-620 nm) has been shown to be highly effective at photo-dissociating CO from COHb *in vitro* (quantum yield 0.5–1) (23 - 26). In a recent study, we demonstrated that in CO-poisoned mice, direct exposure of the lungs to light in the visible spectral range (between 532 and 628 nm) dissociated CO from COHb in the blood passing through the lungs, inducing rapid diffusion of CO into the alveoli and increasing the rate of CO elimination *in vivo*. We also showed that non-invasive phototherapy delivered to murine lungs via an optical fiber placed in the esophagus also increased the rate of CO elimination (27). Phototherapy for patients with CO exposure may provide a significant advance for treating CO poisoning.

The application of non-invasive pulmonary phototherapy to larger animals is challenging, primarily because light needs to penetrate deeper through the tissues to reach the alveoli. Even when directly illuminating the surface of the lung, the poor penetration of the light into the lung parenchyma might limit the ability of pulmonary phototherapy to increase the rate of CO elimination. In the future, the application of pulmonary phototherapy to treat CO poisoning in humans could be more feasible and economical if light sources other than lasers were effective at increasing the CO elimination rate.

The aims of this work were: (1) to develop a model of CO poisoning in anesthetized and mechanically ventilated rats, (2) to assess whether direct pulmonary phototherapy would

decrease COHb half-life in a larger animal model of CO poisoning, (3) to test novel approaches to light delivery in CO-poisoned animals, (4) to study the effect of the combination of pulmonary phototherapy and isocapnic hyperventilation on the CO elimination rate and (5) to investigate the ability of alternative light sources, such as light generated by chemiluminescence, to treat CO poisoning.

MATERIALS AND METHODS

Animals

All animal experiments were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital, Boston, MA. We studied 49 male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA) weighing 454 ± 69 g (mean $\pm$ SD). Rats were anesthetized with intraperitoneal (i.p.) ketamine ($100 \text{ mg}\cdot\text{kg}^{-1}$) and fentanyl ($50 \text{ mcg}\cdot\text{kg}^{-1}$). Following a tracheostomy, rocuronium ($1 \text{ mg}\cdot\text{kg}^{-1}$) was injected i.p. to induce muscle relaxation and rats were mechanically ventilated (Inspira; Harvard Apparatus, Holliston, MA, USA). Volume-controlled ventilation was provided at a respiratory rate of $40 \text{ breaths}\cdot\text{min}^{-1}$, a tidal volume of $10 \text{ ml}\cdot\text{kg}^{-1}$, positive end expiratory pressure (PEEP) of $2 \text{ cmH}_2\text{O}$ and inspired oxygen fraction (FIO_2) of 0.21. Catheters were placed in the carotid artery and jugular vein. Arterial blood pressure, heart rate, body temperature and peak airway pressure were continuously monitored. Continuous i.v. anesthesia and muscle relaxation were provided with fentanyl ($1.2\text{-}2.4 \text{ mcg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$), ketamine ($10\text{-}20 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and rocuronium ($2\text{-}4 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). Ringer's Lactate was infused at a rate of $8\text{-}12 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$.

Carbon monoxide poisoning

Air containing 500 parts per million (ppm) CO was delivered from a gas cylinder (Airgas Inc., Radnor, PA, USA) to the gas inlet of the ventilator. Anesthetized rats were subjected to a median thoracotomy and then breathed 500 ppm CO for 90 minutes as a model of CO poisoning. This degree of CO poisoning did not cause hemodynamic instability and blood lactate levels did not increase. After CO poisoning, rats were treated with either 100% oxygen or air with or without direct lung phototherapy ($n=4$ per group).

To induce a more severe degree of CO poisoning and examine whether pulmonary phototherapy could improve the recovery rate of critically poisoned rats, animals breathed 2000 ppm CO. During the poisoning period, the arterial lactate level was measured every ten minutes (Lactate Plus, Nova Biomedical, Waltham, MA, USA). Treatment by breathing air alone or in combination with direct lung phototherapy ($n=4$ per group) began as soon as blood lactate levels reached a concentration of 7 mmol/L . We set this lactate concentration threshold because in preliminary experiments longer exposures and higher lactate levels resulted in a high rate of mortality *during* the poisoning period, preventing the possibility of evaluating the effect of phototherapy *after* CO poisoning. In experiments examining the effect of phototherapy on arterial lactate levels, fluid resuscitation at $8\text{-}12 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ was provided with 0.9% saline instead of Ringer's Lactate, to avoid interference with lactate measurements.

Direct pulmonary phototherapy

To directly illuminate the lungs, anesthetized rats underwent a median thoracotomy. Light at 532 nm wavelength was generated by an Aura KTP laser (American Medical Systems, Minnetonka, Minnesota, USA) and delivered to both lungs via an optical fiber. The tip of the fiber was placed at 10 cm distance from the lung's surface, so that the area of the light beam was sufficiently wide to illuminate the anterior surface of each lung. The power of the laser was set to illuminate the lungs with an irradiance of $54 \text{ mW}\cdot\text{cm}^{-2}$.

Measurements and calculations

The exhaled CO concentration was measured continuously and recorded once a minute using a CO analyzer (MSA Altair PRO; MSA Safety Inc., Pittsburgh, PA, USA) connected to the expiratory limb of the ventilator. The minute ventilation ($40 \text{ breaths}\cdot\text{min}^{-1}$ and $10 \text{ ml}\cdot\text{kg}^{-1}$ tidal volume, i.e. $400 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was similar in all animals and held constant during the experiment. During the treatment period, the quantity of CO exhaled every minute ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was calculated by multiplying the minute ventilation and the exhaled CO concentration. Then, the quantity of CO eliminated over time was calculated as the sum of the quantities of CO exhaled every minute during that time.

The percentage of COHb in arterial blood was measured during and after CO poisoning. For each measurement, arterial blood ($20 \mu\text{L}$) was mixed with distilled water ($180 \mu\text{L}$) to lyse the red blood cells. The lysate was centrifuged at $15,000 \text{ g}$ for one minute and the supernatant ($50 \mu\text{L}$) was diluted with Dulbecco's phosphate buffered saline (DPBS, $450 \mu\text{L}$). Within one minute after dilution, the absorption spectrum between 500 and 700 nm of the diluted sample was measured using a spectrophotometer (Libra S70, Biochrom, UK). The spectra were analyzed to determine COHb concentration by least-squared fitting of the spectra to the spectrum of standard oxygenated hemoglobin, deoxygenated hemoglobin, COHb and oxidized hemoglobin using Solver software (Excel 2007, Microsoft). For each rat, the rate of decrease of COHb concentration during treatment was determined by fitting the COHb percentage at three to five time points after the beginning of treatment using a single exponential decay equation ($y = e^{-kx}$). The time needed to reduce the initial concentration of COHb by 50% (i.e. COHb half-life or $t_{1/2}$) was calculated as $\text{COHb-}t_{1/2} = \ln(2) \text{ k}^{-1}$.

The power of light reaching the lungs during treatment with phototherapy was measured with a power meter (Thorlabs Inc., Dachau, Germany). In experiments assessing the effect of intrapleural phototherapy, we used fibers with diffusing tips emitting light in all directions. To measure the power emitted by these fibers the power meter was connected to an integrating sphere (Thorlabs Inc., Dachau, Germany) allowing the measurement of power emitted by the diffusing light sources.

Isocapnic hyperventilation

Anesthetized and mechanically ventilated rats were poisoned by breathing 500 ppm CO for 90 minutes and then treated with isocapnic hyperventilation with or without direct pulmonary phototherapy ($n=4$ per group) at 532 nm wavelength and irradiance $54 \text{ mW}\cdot\text{cm}^{-2}$. A five-fold increase in minute ventilation was obtained by ventilating the animals at a respiratory rate of 80 breaths per minute and a tidal volume of $25 \text{ ml}\cdot\text{kg}^{-1}$. Pure

oxygen (100%) was connected to the gas inlet of the ventilator, and CO₂ (approximately 5%) was added to maintain a constant arterial CO₂ partial pressure (PaCO₂), while the end tidal CO₂ (ETCO₂) was continuously measured by a capnometer (PhysioSuite, CapnoScan® End-Tidal CO₂ Monitor, Kent Scientific, Connecticut, USA) and the CO₂ flow rate was adjusted to maintain the ETCO₂ at approximately 33 mmHg.

Intrapleural phototherapy

Two optical fibers with 3.5 cm long diffusing tips emitting light in all directions were placed in the right and left pleural spaces. Insertion of the fibers via intercostal incisions was not possible because the tips of the fibers were rigid and could not be positioned properly in the pleural space because of the small size of the rat's thorax. Therefore, a trans-diaphragmatic approach was used. In anesthetized, mechanically ventilated supine rats, 1.5 cm long incisions were made in the abdominal wall in the right and left hypochondriac regions. After exposing the abdominal cavity and the lower surface of the diaphragm, two 2 mm incisions were made in the diaphragm and one optical fiber was placed in both the right and left pleural spaces. When two optical fibers were connected to the Aura KTP laser outlet by means of a bifurcated optical connector, the power of light emitted by the diffusing tips was very low due to dispersion of light at the coupling sites. To irradiate each lung with sufficient power, the right fiber was connected to the Aura KTP laser and the left fiber was connected to a frequency-doubled Nd:YAG laser (IRIDEX Corp., Mountain View, CA), both lasers generating light at 532 nm. The power of the light emitted by the diffusing optical fibers was 570 (right pleural space) and 450 (left pleural space) mW.

Lung phototherapy using light generated by chemiluminescence

Chemiluminescence is the emission of light as a result of a chemical reaction. We tested whether light generated by chemiluminescence would dissociate CO *in vivo* and increase the rate of CO elimination. We studied green light emitted by Duralume glow-sticks (The Glow Store Inc. Victoria, Canada). The light emission spectrum of Duralume glow-sticks was measured with a spectroradiometer, showing a peak wavelength of 517 nm and a central wavelength of 532 nm (bandwidth (90%) = 110 nm). Duralume contains hydrogen peroxide, which is toxic and therefore cannot be injected into, or make contact with, the lungs. Therefore, in anesthetized rats at thoracotomy, a custom-fabricated clear polyethylene sack was placed around each lung. Each sack had a small catheter allowing the injection of chemiluminescent fluid into the sack. After 90 minutes of CO poisoning, approximately 10 ml of luminescent fluid was aspirated from the glow stick and injected into the polyethylene bags while the animal breathed 100% oxygen. In control animals, the polyethylene bags were filled with an equal volume of saline.

Statistical analysis

Statistical analysis was performed using Sigma Plot 12.5 (SPSS, Inc., Chicago, IL). Data were analyzed using Student's t-test and a one-way ANOVA with post hoc Bonferroni test (two-tailed). A Two Way ANOVA for repeated measurements was used to compare variables over time between groups. Statistical significance was defined as a p value of less than 0.05. All data are expressed as mean $\pm$ SD unless specified otherwise.

RESULTS

Development of a model of CO poisoning in anesthetized rats

In a previous study, we demonstrated that direct pulmonary phototherapy increased the rate of CO elimination in mice (27). To test whether direct phototherapy could increase the rate of CO elimination in an animal that is 18 times heavier than a mouse, we developed a model of CO poisoning in rats. As we previously observed during the poisoning period in mice, the exhaled CO concentration slowly increased, indicating that a large amount of CO was absorbed by the rat at the beginning of poisoning (Figure 1A). Over time, smaller amounts of CO were absorbed as the circulating hemoglobin became progressively saturated with CO. After the poisoning period, rats were treated by breathing either 100% oxygen or room air at a constant minute ventilation. The calculated quantity of CO eliminated during the first 30 minutes of breathing 100% oxygen was significantly greater than that of rats breathing air (3.8 ± 0.1 vs 2.0 ± 0.3 ml•kg⁻¹, $p < 0.001$, Figure 1A). In parallel, blood COHb decreased faster, and COHb- $t_{1/2}$ was shorter in rats breathing 100% oxygen than in rats breathing air (100% oxygen vs room air: 12.0 ± 0.5 vs 53.5 ± 5.1 min, $p < 0.001$, Figure 1B). These results show that the rat model of CO poisoning is suitable for studying the effects of various treatments on the rate of CO elimination.

Phototherapy increases the rate of CO elimination

To test whether phototherapy increases the CO elimination rate, rats were poisoned by breathing 500 ppm CO in air for 90 minutes and then treated by breathing either air or 100% oxygen with or without adding direct lung phototherapy. When CO poisoned rats were treated with either 100% oxygen or room air, pulmonary phototherapy significantly increased the rate of CO elimination, as demonstrated by a greater amount of exhaled CO measured during the first 10 minutes of treatment (100% oxygen vs 100% oxygen plus phototherapy: 2.2 ± 0.1 vs 2.7 ± 0.4 ml•kg⁻¹, $p < 0.05$; air vs air plus phototherapy: 0.9 ± 0.1 vs 1.2 ± 0.1 ml•kg⁻¹, $p = 0.01$, Figure 2A). Concomitantly there was a more rapid reduction of blood COHb levels (COHb- $t_{1/2}$: 100% oxygen vs 100% oxygen plus phototherapy: 12.0 ± 0.5 vs 7.4 ± 1.0 min, $p < 0.001$; air vs air plus phototherapy: 53.5 ± 5.1 vs 38.4 ± 4.6 min, $p = 0.004$, Figure 2B). These results demonstrate that direct pulmonary phototherapy increases the rate of CO elimination in rats breathing either air or 100% oxygen.

Phototherapy improves lactate clearance after CO poisoning

To study whether the more rapid reduction of COHb levels observed with phototherapy could have a salutary effect on oxygen delivery and metabolism after severe CO poisoning, we exposed rats to breathing 2000 ppm CO in air until their blood lactate levels reached a concentration of 7 mmol/L or higher. All animals reached the target lactate concentration within 50 minutes of breathing CO. During the poisoning period the mean arterial pressure (MAP) and the heart rate (HR) decreased from 107 ± 3 to 56 ± 1 mmHg and from 350 ± 15 to 293 ± 12 beats/min respectively (mean $\pm$ SEM, Figure 3A and B). MAP and HR during treatment were not different during treatment in phototherapy-treated rats as compared with rats breathing air alone. The COHb level decreased faster during treatment with, compared to without, phototherapy. The blood lactate concentration was also lower after 20, 40 and 60 minutes of treatment with phototherapy and room air compared to room air alone (20

minutes: 3.7 ± 0.5 vs 5.8 ± 0.4 mmol/L, $p=0.01$; 40 minutes: 1.6 ± 0.2 vs 4.1 ± 0.9 mmol/L, $p=0.009$; 60 minutes: 1.0 ± 0.1 vs 3.2 ± 1.0 mmol/L, $p=0.02$, Figure 3C and D). These results show that CO-poisoned rats breathing air and treated with pulmonary phototherapy have increased lactate clearance.

Combination of pulmonary phototherapy and isocapnic hyperventilation

Hyperventilation increases the rate of CO elimination in dogs and humans after CO poisoning (18, 19). To study whether the combination of isocapnic hyperventilation and pulmonary phototherapy would augment the rate of CO elimination, rats were poisoned by breathing 500 ppm CO in air for 90 minutes and then treated with isocapnic hyperventilation with or without direct lung phototherapy. Despite a slightly lower inspired oxygen concentration (approximately 95% vs 100%) due to the addition of 5% CO₂ to maintain a constant arterial PaCO₂ and avoid hypocapnia, animals treated with isocapnic hyperventilation had a more rapid decrease in blood COHb levels compared to animals treated with 100% oxygen with a normal minute ventilation rate (COHb- $t_{1/2}$: 7.1 ± 0.4 vs 12.0 ± 0.5 min, $p<0.001$). The combination of direct pulmonary phototherapy and isocapnic hyperventilation increased the rate of CO elimination as compared with isocapnic hyperventilation alone, as demonstrated by a larger amount of CO eliminated during the first ten minutes of treatment (isocapnic hyperventilation plus phototherapy vs isocapnic hyperventilation alone: 6.1 ± 0.6 vs 4.2 ± 0.3 ml•kg⁻¹, $p=0.001$, Figure 4A) and a decreased COHb- $t_{1/2}$ (5.2 ± 0.5 vs 7.1 ± 0.4 min, $p=0.002$). These results show that increased minute ventilation increases the rate of CO elimination in rats. Moreover, combining pulmonary phototherapy and isocapnic hyperventilation with 95% oxygen can further increase the rate of CO elimination.

Intrapleural phototherapy

To treat CO poisoning with direct lung irradiation, without the need for a thoracotomy, we tested an intrapleural approach to light delivery to the lungs, via two optical fibers with diffusing tips inserted in the right and left pleural spaces. After 90 minutes of poisoning with 500 ppm CO in air, rats were treated with 100% oxygen with or without intrapleural phototherapy. Compared with rats treated with 100% oxygen alone, intrapleural phototherapy combined with 100% oxygen was associated with a greater quantity of CO elimination during the first ten minutes of treatment (2.4 ± 0.2 vs 2.1 ± 0.1 ml•kg⁻¹, $p=0.009$) and a shorter COHb- $t_{1/2}$ (9.8 ± 0.5 vs 11.7 ± 0.3 min, $p<0.001$) (Table 1). These results demonstrate that intrapleural phototherapy can increase the rate of CO elimination in a CO poisoned rat breathing 100% oxygen.

Chemiluminescence-generated lung phototherapy

To apply pulmonary phototherapy to CO poisoning in humans, it would be more feasible and economical to use a light source other than lasers. We therefore tested the effect of green light, generated by a chemiluminescent reaction, on CO elimination rate in CO-poisoned rats. The amount of CO exhaled during the first ten minutes of treatment was greater in rats treated with 100% oxygen and chemiluminescence-generated phototherapy compared to rats breathing 100% oxygen alone (2.4 ± 0.1 vs 2.1 ± 0.1 ml•kg⁻¹, $p=0.018$), and the COHb- $t_{1/2}$ was significantly shorter (10.1 ± 0.6 vs 11.5 ± 0.3 min, $p=0.011$) (Table 1). These results show

that providing green light generated by a chemiluminescent reaction in both pleural spaces can increase the rate of CO elimination, suggesting that protected chemiluminescent light sources might be used to irradiate the lungs.

DISCUSSION

In a previous study, we showed that in CO poisoned mice, laser-generated visible light dissociated CO from hemoglobin in the pulmonary vasculature, enhancing CO diffusion from blood to the alveoli and increasing the rate of CO elimination (27). The application of pulmonary phototherapy to treat CO poisoning in humans is challenging, primarily because of the large size of the human lung. Moreover, a non-invasive approach to light delivery needs to be developed, to allow treatment of CO poisoned patients as early as possible in the field. For this purpose, alternative light sources, fibers and delivery methods might be used to reach the lungs less invasively.

In this work we report novel aspects of pulmonary phototherapy as compared to our previous study in mice. First, we tested phototherapy in a larger animal model, specifically in rats weighing 18 times more than mice (~ 450 g vs 25 g). Second, we show that pulmonary phototherapy improved a clinical outcome (lactate clearance) *after* CO poisoning. In our previous work we were only able to demonstrate a clinical benefit by applying phototherapy *during* CO poisoning, because severe CO poisoning in mice was an acute lethal event before any effective treatment could be instituted. Third, we assessed the effect of the combination of pulmonary phototherapy and hyperventilation. Fourth, we tested a less invasive approach to deliver light to the lungs via intrapleural optical fibers. Finally, we tested chemiluminescence as an alternative source of light to treat CO poisoning.

As a first step toward applying this novel therapy to humans, we applied pulmonary phototherapy for treating CO poisoning to exposed lungs of anesthetized rats. As we observed in mice, direct pulmonary phototherapy reduced blood COHb- $t_{1/2}$ by 40% as compared to treatment with oxygen alone, and reduced COHb- $t_{1/2}$ by 30% as compared to treatment with air alone.

During severe CO poisoning, the presence of high levels of COHb reduces the blood oxygen carrying capacity. CO also increases hemoglobin's oxygen affinity, reducing the P_{50} of hemoglobin and impairing oxygen release to tissues (28, 29). Oxygen delivery is particularly compromised in the earliest phase of resuscitation, when COHb levels are high, and lactic acidosis occurs because of reduced oxygen availability for metabolism (30 - 32). In this early phase of resuscitation, oxygen tanks may be unavailable and the only possible therapeutic intervention may be to physically remove the patient from continued CO exposure. To test whether pulmonary phototherapy could have a beneficial effect in the earliest phase of resuscitation when oxygen is not available, rats were severely poisoned by breathing CO at 2000 ppm and then treated by breathing air with or without pulmonary phototherapy. After breathing CO at 2000 ppm for 50 minutes, 60% of circulating hemoglobin was saturated with CO and the plasma lactate concentration increased to approximately 7 mmol/L. The combination of air breathing and phototherapy was associated with a more rapid reduction in COHb levels, resulting in improved oxygen delivery and

more rapid plasma lactate clearance (Figure 3). Development of non-invasive portable devices generating light to treat CO poisoning with pulmonary phototherapy, at the site where poisoning occurs, could play a critical role in removing CO from the blood in the earliest phase of resuscitation.

Fisher et al. showed that CO elimination during treatment with oxygen can be improved by increasing the minute ventilation in dogs and humans (18, 19). The combination of increased minute ventilation and increased cardiac output during mild exercise also improved the CO elimination rate in young healthy subjects (20). In this study, isocapnic hyperventilation increased the rate of CO elimination in rats, and adding pulmonary phototherapy to isocapnic hyperventilation further reduced COHb- $t_{1/2}$ by 27% (Figure 4). Isocapnic hyperventilation and phototherapy act by two different and cooperative mechanisms. Hyperventilation increases the rate of CO elimination by reducing the alveolar CO concentration and increasing the blood to alveolar CO concentration gradient (18). Phototherapy efficiently dissociates CO from COHb, reducing the affinity of CO for hemoglobin by increasing the rate of CO release from hemoglobin. Based on these considerations, hyperventilation and phototherapy can be combined to eliminate CO more rapidly from CO poisoned patients.

In this study, we tested phototherapy at 532 nm delivered to the lungs directly via intrapleural optical fibers, allowing direct lung illumination while avoiding a thoracotomy. The CO elimination rate was improved by intrapleural phototherapy, although the effect was smaller than the effect measured produced by providing direct exposure of the lungs to light. The optimal CO dissociation rate that would be of clinical significance in humans with CO poisoning is not known. Previous studies in humans have shown that hyperbaric oxygen administration (HBO), which reduces COHb- $t_{1/2}$ by 50-60% when compared to treatment with normobaric oxygen (NBO), decreased the long-term neurologic sequelae of CO poisoning (33). Although multiple mechanisms may account for the clinical improvement associated with HBO, a similar reduction in COHb- $t_{1/2}$ using phototherapy would also be expected to be clinically significant. In addition, because pulmonary phototherapy could be administered more promptly than HBO (which requires transport to a facility that can offer this treatment), a smaller reduction in COHb- $t_{1/2}$ might also be beneficial, as the total time required to re-establish oxygen delivery to the tissue would be reduced. In our study in rats, COHb- $t_{1/2}$ was reduced by 16% when pulmonary phototherapy at 532 nm was delivered via two intrapleural optical fibers. To improve the CO dissociation, multiple intrapleural optical fibers delivering light at a longer wavelength (and thus with deeper tissue penetration) may be used. Further studies in larger animals to test this hypothesis will be required before translating pulmonary phototherapy to humans. Employing multiple intrapleural optical fibers to increase the irradiated surface area of the lung combined with the use of light with a longer wavelength (and greater tissue penetration (34)) may result in a greater increase in the rate of CO elimination.

To irradiate a larger surface area of the lungs, we hypothesized that partially enveloping the lungs with a clear sack containing a light-emitting fluid in the pleural spaces would result in an increased CO elimination rate. Chemiluminescence-generated light delivered to the lungs in this manner increased the rate of CO elimination. Our results suggest that intrapleural

devices, allowing the injection of high-energy light-emitting fluids via bilateral thoracostomy tubes, may be designed to treat CO poisoned patients.

Most chemiluminescent reactions require hydrogen peroxide, which is toxic to the lungs. In contrast to chemiluminescence, phosphorescence involves the emission of light from a substance exposed to radiation and light emission persists after the exciting radiation has been removed. Generation of light by phosphorescence does not involve any chemical reaction, and would be expected to be less toxic than chemiluminescence. In the future, phosphorescent particles might be used as an alternative light source for treating CO poisoning. Another option may be the development of light-emitting nano-particles, which could be injected into the blood stream and would deliver the light directly to red blood cells. When non-toxic and soluble agents emitting visible light become available, we envision their delivery to the lungs via intravascular injection or via aerosol. In this way, a large portion of the alveolar-capillary membrane will be treated, and COHb will be irradiated in the pulmonary capillaries where gas exchange occurs.

This study has some limitations. First, phototherapy is highly efficient at increasing the CO elimination rate in rats when administered at thoracotomy (38% COHb- $t_{1/2}$ reduction), while using intrapleural optical fibers and chemiluminescence generated phototherapy produced a smaller reduction in COHb- $t_{1/2}$ (16% and 12% respectively). Second, the improved lactate clearance in the severe model of CO poisoning was obtained by using direct pulmonary phototherapy; we did not test the effect of less invasive approaches to light delivery on clinical outcomes in this model. Third, there is evidence that the long-term neurocognitive deficits after CO poisoning are caused by CO-mediated mitochondrial dysfunction, free radical generation and immune system activation, rather than by the increased COHb levels per se. Further studies will be required to assess whether early treatment with non-invasive phototherapy might prevent long-term neurologic sequelae.

In conclusion, as a first step in furthering the application of pulmonary phototherapy from mice to humans, we report that pulmonary phototherapy increased the rate of CO elimination in rats breathing either air or oxygen, and increased plasma lactate clearance in the early phase of resuscitation of critically poisoned rats breathing air. Laser-generated pulmonary phototherapy delivered to the lungs of rats via intrapleural optical fibers as well as phototherapy generated by a chemiluminescent reaction in the pleural spaces improved the CO elimination rate. The development of novel devices to less invasively apply pulmonary phototherapy to larger animals and humans may provide a new therapeutic strategy for the treatment of CO poisoned patients.

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Figure 1.

Rat model of CO poisoning. Anesthetized and mechanically ventilated rats were poisoned with 500 ppm CO for 90 minutes and then treated with either air ($n = 4$) or 100% oxygen ($n = 4$). (A) Inhaled and exhaled CO concentrations were continuously measured during the poisoning and washout periods. The area between the inhaled and exhaled CO curves during the poisoning period reflects the quantity of CO absorbed. The areas under the exhaled CO concentration curves during treatment represent the amount of CO eliminated when breathing either air or 100% oxygen. (A inset) In the first 30 minutes of the washout period, rats breathing oxygen eliminated a larger amount of CO, $p < 0.001$, Student's t -test. (B) Arterial COHb levels decreased faster when rats breathed 100% oxygen compared with air breathing and (B inset) COHb- $t_{1/2}$ was significantly shorter ($p < 0.001$, Student's t -test). All data represent mean $\pm$ SD.

Figure 2.

Phototherapy increases the rate of CO elimination in rats treated with either 100% oxygen or air. After 90 minutes of poisoning at 500 ppm CO, rats were treated with either 100% oxygen or air with or without lung phototherapy (n = 4 per group). Exhaled CO concentration at the beginning of treatment with both air and 100% oxygen was higher when pulmonary phototherapy was added (A). A larger amount of CO was eliminated during the first 10 minutes of treatment with pulmonary phototherapy compared to treatment by breathing air or oxygen alone (A inset), Student's t-test. (B) Arterial COHb levels decreased

more rapidly when pulmonary phototherapy was added to both air and 100% oxygen breathing, and COHb- $t_{1/2}$ was significantly shorter, Student's t-test (B inset). Data represent mean $\pm$ SEM in panel A and mean $\pm$ SD in panel B.

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Figure 3.

Phototherapy improves lactate clearance after CO poisoning. Rats were poisoned at 2000 ppm CO to obtain an arterial lactate level of 7 mmol/L or higher. After poisoning, rats were treated with air with or without direct lung phototherapy at 532 nm ($n = 4$ per group). (A) Mean arterial pressure (mean $\pm$ SEM) was slightly but not significantly higher during treatment with air and phototherapy as compared to air breathing alone. (B) Heart rate (mean $\pm$ SEM) was not different in the two treatment groups. (C) Arterial COHb levels (mean $\pm$ SD) decreased more rapidly when pulmonary phototherapy was added to air breathing. (D) Lactate levels (mean $\pm$ SEM) decreased faster in rats treated with air and phototherapy as compared to rats breathing air alone ($p < 0.05$ vs. air breathing, two-way ANOVA for repeated measurements).

Figure 4.

Pulmonary phototherapy increases the rate of CO elimination in rats treated with isocapnic hyperventilation. After 90 minutes of poisoning breathing 500 ppm CO, rats were treated with isocapnic hyperventilation with or without lung phototherapy (n = 5 per group). Exhaled CO concentration during treatment with isocapnic hyperventilation and phototherapy was higher than treatment with isocapnic hyperventilation alone (A), and a larger amount of CO was eliminated during the first 10 minutes of treatment (A inset), $p = 0.001$, Student's t-test. (B) Arterial COHb levels decreased more rapidly when pulmonary phototherapy was added to isocapnic hyperventilation, and (B inset) COHb- $t_{1/2}$ was significantly shorter, Student's t-test. All data represent mean $\pm$ SD.

Intrapleural and chemiluminescence-generated phototherapy increase the CO elimination rate

Table 1

Anesthetized and mechanically ventilated rats were poisoned with 500 ppm CO for 90 minutes and then treated with 100% oxygen alone or combined with phototherapy delivered to the lungs via intrapleural optical fibers (n=5) or generated by a chemiluminescent reaction (n=3). During the first 10 minutes of treatment the amount of CO eliminated by rats treated with both intrapleural and chemiluminescence-generated phototherapy was greater than in rats breathing 100% oxygen alone. Arterial COHb levels decreased more rapidly during treatment with 100% oxygen plus intrapleural or chemiluminescence-generated phototherapy when compared to breathing 100% oxygen alone, and COHb-t_{1/2} was significantly shorter. All data represent mean ± SD.

	100% O ₂ (n=5)	100% O ₂ + intrapleural phototherapy (n=5)	P
COHb-t _{1/2} (min)	11.7 ± 0.3	9.8 ± 0.5	<0.001
10 min CO elimination (ml/kg)	2.1 ± 0.1	2.4 ± 0.2	0.009

	100% O ₂ (n=4)	100% O ₂ + chemiluminescence (n=3)	P
COHb-t _{1/2} (min)	11.5 ± 0.6	10.1 ± 0.6	0.011
10 min CO elimination (ml/kg)	2.1 ± 0.1	2.4 ± 0.1	0.018