CARBON MONOXIDE POISONING

Phototherapy and extracorporeal membrane oxygenation facilitate removal of carbon monoxide in rats

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Inhaled carbon monoxide (CO) displaces oxygen from hemoglobin, reducing the capacity of blood to carry oxygen. Current treatments for CO-poisoned patients involve administration of 100% oxygen; however, when CO poisoning is associated with acute lung injury secondary to smoke inhalation, burns, or trauma, breathing 100% oxygen may be ineffective. Visible light dissociates CO from hemoglobin. We hypothesized that the exposure of blood to visible light while passing through a membrane oxygenator would increase the rate of CO elimination in vivo. We developed a membrane oxygenator with optimal characteristics to facilitate exposure of blood to visible light and tested the device in a rat model of CO poisoning, with or without concomitant lung injury. Compared to ventilation with 100% oxygen, the addition of extracorporeal removal of CO with phototherapy (ECCOR-P) doubled the rate of CO elimination in CO-poisoned rats with normal lungs. In CO-poisoned rats with acute lung injury, treatment with ECCOR-P increased the rate of CO removal by threefold compared to ventilation with 100% oxygen alone and was associated with improved survival. Further development and adaptation of this extracorporeal CO photo-removal device for clinical use may provide additional benefits for CO-poisoned patients, especially for those with concurrent acute lung injury.

INTRODUCTION
Carbon monoxide (CO) is a toxic, colorless, and odorless gas released from incomplete combustion of carbon-containing fuel (1). CO intoxication is a leading cause of poisoning-related deaths and results in more than 50,000 visits to emergency departments in the United States each year (2, 3). When inhaled, CO competes with oxygen and avidly binds to hemoglobin in the pulmonary capillaries, reducing arterial blood oxygen saturation (4). The resulting decrease in oxygen delivery to peripheral tissues, particularly to the brain and the heart, is a key pathophysiological mechanism underlying CO toxicity (5–8). CO can also cause direct tissue damage by binding to other homeostatic proteins including cytochromes and myoglobin (9, 10). Because oxygen competes with CO for binding to hemoglobin, administration of normobaric 100% oxygen (NBO) is the mainstay of CO poisoning treatment (11). Hyperbaric oxygen (HBO), which acts by increasing the partial pressure of oxygen (PO2), further increases the rate of CO elimination in patients with normal lungs (12, 13).

Exposure to CO is often associated with inhalation of other chemicals and particulates (14), which can damage the airways and alveoli, resulting in acute lung injury and respiratory failure (15). In soldiers and firefighters, trauma and burns may contribute to acute respiratory distress syndrome (ARDS) (16).

The rate of CO removal is defined as the time required to reduce blood CO hemoglobin (COHb) by 50% [COHb half-life (COHb-t1/2)]. In healthy human volunteers exposed to CO and treated with 100% oxygen, COHb-t1/2 is 90 min (17). However, COHb-t1/2 has been reported to be as long as 148 min in CO-poisoned patients treated with 100% oxygen, suggesting that impaired CO elimination via injured lungs might often be present (18, 19). Whenever CO poisoning is associated with impaired gas exchange in the lungs, treatment with either NBO or HBO might be less effective or even noxious (20).

Two case reports described patients with severe CO poisoning and respiratory and cardiovascular failure, who were successfully treated with extracorporeal membrane oxygenation (ECMO). The circulation of venous blood through an artificial membrane allows oxygenation of the blood and removal of CO when the lungs are injured (21). Haldane and Smith (22) first demonstrated that visible light dissociates CO from hemoglobin, without affecting the bond between oxygen and hemoglobin (22–25). In two recent studies, we demonstrated that irradiation of lungs with visible light dissociates CO from hemoglobin in the pulmonary circulation and increases the rate of CO elimination in mice and rats exposed to CO (26, 27). We hypothesized that visible light might dissociate CO from hemoglobin in blood passing through an extracorporeal membrane oxygenator, facilitating CO elimination and providing an effective treatment for CO poisoning, especially in the setting of impaired respiratory function. In this study, we report the development of a device that combines phototherapy with a membrane oxygenator (“CO photo-remover”). We show the effectiveness of veno-venous extracorporeal CO removal, at a low blood flow rate, using phototherapy for the treatment of CO poisoning in rats with healthy lungs and in rats with acute lung injury.

RESULTS
Development of an extracorporeal CO photo-remover
To create a miniature membrane oxygenator with optimal characteristics for oxygen transfer, CO removal, and venous blood light exposure, we used a microporous polypropylene membrane for gas exchange and...
a clear plexiglass case to allow light penetration. The polypropylene membrane was obtained from a disassembled cardiopulmonary bypass oxygenator and cut into 7 cm x 7 cm sections (fig. S1). Eight sections were placed on top of each other, with each layer rotated 90° relative to the one below. The edges of the membranes were sealed with silicone rubber adhesive and enclosed in a clear plexiglass case with four openings to serve as blood and gas inlets and outlets. The configuration of the device was such that two sealed compartments were obtained: a compartment for blood flowing around the hollow fibers, and a compartment for oxygen flowing into the hollow fibers. The size of the chamber containing the hollow fibers and the blood was 6 cm wide, 6 cm long, and 4 mm high. Within the chamber, the priming volume for blood was 4 ml, whereas the remaining volume was occupied by the hollow fibers. The surface area for gas exchange was 0.05 m², and the total surface area for phototherapy was 72 cm².

Oxygenating performance of the CO photo-remover

To determine the in vitro oxygenating performance of the CO photo-remover, deoxygenated whole blood was circulated through the CO photo-remover at various flow rates, while the device was “ventilated” with 100% oxygen. In commercially available ECMO devices, PO₂ in blood exiting the membrane lung is between 400 and 500 mmHg (28). PO₂ of the blood entering and exiting the CO photo-remover was 35.5 ± 11.9 mmHg and 482 ± 72 mmHg, respectively (P < 0.0001, Fig. 1A). The actual and the maximum oxygen transfer were calculated according to standard formulae (see Methods). The actual oxygen transfer was similar to the maximum oxygen transfer (R² = 0.99, slope = 0.85, P < 0.0001; Fig. 1B), and the oxygen transfer increased linearly with increasing blood flow rate (R² = 0.78, P < 0.0001; Fig. 1C). These results indicate that the CO photo-remover has an oxygen transfer performance equivalent to a commercial device used for cardiopulmonary bypass and ECMO.

Extracorporeal removal of CO using phototherapy in CO-poisoned rats

To determine whether the CO photo-remover was able to increase the rate of CO elimination from blood in vivo, we tested the device in a rat model of CO poisoning (Fig. 3A). Anesthetized and mechanically ventilated rats were poisoned by breathing 2000 parts per million (ppm) CO in air for 30 min. All animals underwent veno-venous extracorporeal blood circulation with blood flow rate ranging from 50 to 100 ml/kg per minute [which corresponds to approximately 15 to 30% of the rat’s cardiac output (29)] and were treated by breathing 100% oxygen, whereas the CO photo-remover was provided with (i) neither gas nor light (control), (ii) gas flow (95% O₂ and 5% CO₂) but no phototherapy (ECCOR), (iii) gas flow and phototherapy with combined green and blue light (ECCOR-P-Green/Blue), and (iv) gas flow and phototherapy with red light (ECCOR-P-Red). Light was generated by four LEDs on each side of the device (fig. S1).

**Fig. 1. Oxygenating performance of the CO photo-remover.** (A) PO₂ entering and exiting the CO photo-remover. P < 0.0001, paired t test. (B) Relationship between the maximum oxygen transfer and the actual oxygen transfer across the CO photo-remover. R² = 0.99, slope = 0.85, P < 0.0001. (C) Relationship between oxygen transfer and blood flow rates. R² = 0.78, P < 0.0001.
In control animals, no CO was eliminated by the device (Fig. 3B). Because the CO eliminated by the CO photo-remover during phototherapy (ECCOR-P-Green/Blue and ECCOR-P-Red) was higher than the CO eliminated without phototherapy (ECCOR-only gas flow, Fig. 3B), there was less CO remaining to be exhaled from the lungs of treated animals compared to control rats (Fig. 3C). Arterial blood COHb decreased faster in animals treated with ECCOR and gas flow as compared to controls (Fig. 3D), and COHb-t1/2 was reduced by 28% (12.8 ± 0.6 min versus 17.7 ± 1.2 min, P < 0.0001; Fig. 3E). Addition of phototherapy to the CO photo-remover further increased the rate of COHb reduction (Fig. 3C). Irradiation of the device with green and blue light produced a 47% reduction in COHb-t1/2 compared to controls (9.4 ± 0.8 min versus 17.7 ± 1.2 min, P < 0.0001) and a 27% reduction in COHb-t1/2 compared to ECCOR with gas but without light (9.4 ± 0.8 min versus 12.8 ± 0.6 min, P < 0.001). Red light produced a 53% reduction in COHb-t1/2 compared to controls (8.6 ± 0.5 min versus 17.7 ± 1.2 min, P < 0.0001) and a 33% reduction in COHb-t1/2 compared to ECCOR with gas but without light (8.6 ± 0.5 min versus 12.8 ± 0.6 min, P < 0.001; Fig. 3E). These results...
show that the extracorporeal removal of CO using phototherapy (ECCOR-P) increases the rate of blood CO elimination in vivo.

**Effect of ECCOR using phototherapy on blood lactate clearance and tissue oxygenation after severe CO poisoning**

To determine whether the faster reduction of COHb observed with the combination of ECCOR and phototherapy has a beneficial effect on tissue oxygenation after CO poisoning, we developed a more severe model of CO poisoning. Rats were anesthetized and poisoned by breathing 2000 ppm CO in air for 45 min and developed tissue hypoxia and lactic acidosis. During CO poisoning, mean arterial pressure (MAP) and heart rate (HR) decreased and increased, respectively (fig. S3). In all animals, arterial COHb similarly increased to 55 to 60% at the end of the CO poisoning period (Fig. 4A), whereas venous \( P_O2 \) decreased from 35 to 45 mmHg to 10 to 15 mmHg (Fig. 4B) and lactate concentration increased to 7 to 8 mM (Fig. 4C).

After poisoning, animals were treated by breathing air so as to mimic a situation of reduced arterial oxygenation, as may occur in a patient with lung damage and impaired gas exchange. All animals were treated with veno-venous extracorporeal blood circulation with blood flow rates ranging from 50 to 100 ml/kg per minute. In control animals, the device was provided with neither gas flow nor phototherapy. After poisoning, arterial blood COHb decreased faster in animals treated with ECCOR and gas flow as compared to controls. Addition of phototherapy to the CO photo-remover further increased the rate of COHb reduction: At each time point, arterial blood COHb in each group was different from all other groups (Fig. 4A). In particular, treatment with ECCOR and gas flow, but no phototherapy, reduced COHb-\( t_{1/2} \) by 44% (54.9 ± 2.7 min versus 31.2 ± 1.5 min, \( P < 0.0001 \)). Treatment with ECCOR, gas flow, and combined green and blue light (ECCOR-P-Green/Blue) reduced COHb-\( t_{1/2} \) by 67% compared to control animals (54.9 ± 2 min versus 18.0 ± 0.7 min, \( P < 0.0001 \)). Treatment with ECCOR gas flow and red light (ECCOR-P-Red) reduced COHb-\( t_{1/2} \) by 79% compared to controls (54.9 ± 2 min versus 11.6 ± 1.4 min, \( P < 0.0001 \)) and by 35% compared to treatment with ECCOR-P-Green/Blue (18.0 ± 0.7 min versus 11.6 ± 1.4 min, \( P = 0.014 \)) (Fig. 4A, inset).

At the beginning of the treatment period, the elimination of CO from the lungs of CO-poisoned animals was similar in all four groups (time 0 in Fig. 4D). In later phases of the treatment period (10 to 60 min after the beginning of the treatment), the CO exhaled from the lungs of control animals was higher than in treated animals, as a greater amount of COHb remained in the circulation and more CO was available to be removed by the lungs. The elimination of CO from the CO photo-remover was greater using ECCOR with red light than with ECCOR alone or with ECCOR with green and blue light (Fig. 4E).

Animals treated with ECCOR-P-Red or ECCOR-P-Green/Blue had a faster return of venous \( P_O2 \) to baseline (Fig. 4B) compared to control animals (for which neither gas flow nor phototherapy was applied to the device). Lactate clearance was also faster in
ECCOR-P–treated animals (Fig. 4C), resulting in a more rapid return of base excess to baseline values and correction of metabolic acidosis (Fig. 4F). No difference in MAP or HR was found among groups at baseline, during CO poisoning, or during treatment (fig. S3).

Together, these results show that, in CO-poisoned rats breathing room air, veno-venous ECCOR-P markedly increases the rate of CO elimination and that red light is more effective than the combination of green and blue light in removing CO from the blood of CO-poisoned rats. The faster removal of CO from circulating blood is associated with improved tissue oxygenation, as well as faster clearance of systemic lactate and correction of metabolic acidosis.

**Treatment with ECCOR using phototherapy after CO poisoning and acute lung injury**

In rats that were poisoned with CO, the use of ECCOR-P-Red doubled the rate of CO elimination compared to rats breathing 100% oxygen and produced a fivefold increase in the rate of CO removal in rats breathing room air. We hypothesized that ECCOR-P-Red would be particularly beneficial in a situation of limited gas exchange, such as may occur in patients with acute lung injury. We used intravenous injection of oleic acid to produce a rat model of acute lung injury and impaired gas exchange (Fig. 5A). This model has a high degree of reproducibility, and the histopathological and physiological changes caused by oleic acid are similar to those seen in patients with ARDS (30). After oleic acid injection, animals were poisoned with 2000 ppm CO for 45 min. All of the control animals, treated with 100% oxygen but no extracorporeal circulation, died within 46 min after the initiation of treatment. In contrast, all animals treated with 100% oxygen ventilation and ECCOR-P-Red (initiated immediately after the poisoning) survived until the endpoint of the study (60 min after the initiation of treatment; Fig. 5B). The severity of the acute lung injury and the CO poisoning was comparable in the two groups: (i) the
ratio between PO$_2$ and FiO$_2$ (fraction of inspired oxygen) (P/F ratio) and the respiratory system compliance ($C_{rs}$) were reduced at the end of the CO poisoning (and beginning of treatment) in both groups (Fig. 5C); (ii) arterial pH, PO$_2$, and O$_2$Hb similarly decreased during lung injury and CO poisoning (Fig. 5, D to F). Partial pressure of carbon dioxide (P$_{CO_2}$) initially increased due to lung injury and then decreased during CO poisoning, likely due to reduced CO$_2$ production in the setting of tissue hypoxia (Fig. 5G); and (iii) before treatment, MAP, HR, and the arterial blood lactate concentration were similar in both groups (Fig. 5, H to J).

In oleic acid–treated and CO-poisoned rats ventilated with 100% oxygen, the circulating COHb decreased faster in rats treated with ECCOR-P-Red than in those treated with 100% oxygen alone (COHb$_{t_{1/2}}$: 11.6 ± 2.4 min versus 28.5 ± 3.5 min, $P = 0002$; Fig. 5K). Compared to control rats, rats treated with ECCOR-P-Red had transiently higher pH and O$_2$Hb and lower P$_{CO_2}$ during the treatment period. These results show that, in rats with acute lung injury and CO poisoning, treatment with the veno-venous ECCOR-P markedly increased the rate of CO elimination and improved overall survival.

**In vivo efficiency of ECCOR-P at various blood flow rates**

To assess whether the efficacy of CO removal by veno-venous ECCOR-P is altered by changing the rate of blood circulating through the device, rats were poisoned with 2000 ppm CO in air and then treated with 100% oxygen and ECCOR-P-Red at different blood flow rates. Treatment with ECCOR-P-Red increased the rate of CO elimination at blood flows ranging from 10 to 85 ml/kg per minute (Fig. 6A). The relationship between COHb$_{t_{1/2}}$ and the veno-venous extracorporeal blood flow rate is described by an exponential decay curve \[ Y = (Y_0 - \text{Plateau}) \cdot \exp(-K \cdot X) + \text{Plateau} \] \[ (R^2 = 0.99; \text{Fig. 6A}). \]

When the blood flow was greater than 20 ml/kg per minute [which corresponds to about 5 to 10% of the rat’s cardiac output (29)], a CO removal plateau was reached (COHb$_{t_{1/2}}$ plateau = 8.6 min), indicating that a further increase in the blood flow rate did not increase the rate of CO elimination.

To investigate whether ECCOR-P is effective at a wide range of blood flow rates in animals breathing lower concentrations of oxygen, rats were poisoned with 2000 ppm CO in air and then treated by breathing air instead of 100% oxygen. The rats were treated with ECCOR-P-Red at different blood flow rates. ECCOR-P-Red was effective at blood flows ranging from 10 to 85 ml/kg per minute, with a COHb$_{t_{1/2}}$ plateau of 12.4 min ($R^2 = 0.97$; Fig. 6B).

At each blood flow rate, the performance of the CO photo-remover was assessed by measuring the amount of CO in the blood entering the device and the amount of CO in the gas effluent. At blood flow rates greater than 20 ml/min, about 10% of the CO entering the device was eliminated. In contrast, at lower blood flow rates, up to 35% of the CO entering the device was eliminated (Fig. 6C). These results show that the ECCOR-P is highly effective at a wide range of blood flow rates and that, at low blood flow rates, more CO is eliminated per unit of blood entering the CO photo-remover.

**DISCUSSION**

In this study, we report the development of a device (CO photo-remover) for veno-venous ECCOR-P in rats. In vitro testing showed that red light was more effective than green and blue light in enhancing CO removal from human blood. We used a rat model of CO poisoning to demonstrate the ability of ECCOR-P to increase the rate of CO elimination in vivo. Compared to CO-poisoned animals treated by breathing 100% oxygen alone, the addition of ECCOR with gas flow but without phototherapy increased the rate of CO elimination by 28%. By adding red light to ECCOR with gas flow, the CO elimination rate was increased by 53%, which is similar to the relative reduction in COHb$_{t_{1/2}}$ that occurs when CO-poisoned patients with healthy lungs are treated with HBO instead of NBO (13).

When CO-poisoned rats were treated by breathing air, the addition of ECCOR without light increased the rate of CO elimination by 40%. The addition of red light to ECCOR produced a fivefold increase in the rate of CO elimination, with an improvement in tissue oxygenation and blood lactate clearance, and a decrease in metabolic acidosis. In rats with lethal CO poisoning and acute lung injury, treatment with ECCOR-P-Red increased the rate of CO elimination.
increased permeability of the alveolar-capillary membrane (37 occur as a consequence of CO-induced cardiogenic shock (31) and ventilation for management of ARDS (35) require emergent intubation for airway protection and mechanical injuries. Thermal and chemical damage to airways and alveoli may absorb coefficient for green and blue light is higher than for red be higher than during red phototherapy, because the hemoglobin green or blue phototherapy, the temperature of the blood tended to is the likely explanation for the increased rate of dissociation of CO from hemoglobin observed in this study (34). Note, when using green or blue phototherapy, the temperature of the blood tended to be higher than during red phototherapy, because the hemoglobin absorption coefficient for green and blue light is higher than for red light (35). In addition to enabling phototreatment of a greater amount of blood due to increased penetration, the use of red phototherapy may prevent blood from overheating.

CO poisoning may occur together with a spectrum of acute lung injuries. Thermal and chemical damage to airways and alveoli may require emergent intubation for airway protection and mechanical ventilation for management of ARDS (15). Pulmonary edema may occur as a consequence of CO-induced cardiogenic shock (36) and increased permeability of the alveolar-capillary membrane (37, 38). Two case reports described CO poisoning and lung injury caused by a mixture of formic and sulfuric acid (39, 40). In patients who have been exposed to fires and explosions or have experienced trauma, ARDS secondary to burns and lung contusion from blast injury may also be present (16). Whenever a patient’s clinical condition is complicated by impaired ventilation and decreased gas exchange, HBO and NBO may be ineffective approaches to increasing the rate of CO removal from hemoglobin and may worsen lung injury due to oxygen toxicity (41). In this study, we investigated the effect of ECCOR-P in a rat model of lung injury. Oleic acid–induced lung injury was consistently reproducible, and all animals developed acute lung injury with similar impairment in gas exchange, as demonstrated by the similar increase in Pco2 and decrease in Po2. CO elimination in animals with lung injury was impaired as compared to animals with normal lungs. In the setting of hypoxia and acidosis, treatment with ECCOR-P increased the rate of CO elimination threefold, improved oxygenation as well as the metabolic state, and improved survival. These results suggest that the ECCOR-P may represent a unique alternative treatment in patients with lung injury.

The ECMO of blood at high flow rates for respiratory and cardiovascular support requires large bore cannulas and systemic anticoagulation. In contrast, hemodialysis and CO2 removal may be accomplished using lower blood flow rates, less invasive double-lumen venous catheters, and regional anticoagulation (42–44). In this study, all animals received systemic anticoagulation with heparin to prevent clotting in the CO photo-remover and allow reuse of the device in multiple experiments. In CO-poisoned patients requiring short-term treatment with disposable devices, systemic anticoagulation might be avoided.

In this study, we used a device with a priming volume that was about 10% of the total blood volume (45) and we found that the extracorporeal photo-removal of CO was highly efficient at venous blood flow rates as low as 10 ml/kg per minute [which corresponded to about 5% of the rat’s cardiac output (29)]. In fact, at high blood flow rates, only about 10% of the CO entering the CO photo-remover was eliminated. In contrast, presumably because of an increase in the blood transit time, at low blood flow rates, the performance of the CO photo-remover was improved, with 35% of the CO entering the CO photo-remover being eliminated. On the basis of these findings, we anticipate that a veno-venous extracorporeal circulation at 250 ml/min, using a device with a priming volume of 250 to 300 ml, will be effective at treating an adult CO-poisoned patient with a blood volume of 5 liters and cardiac output of 5 liters/min. To both minimize the thickness of the blood layer (and thereby allow greater light penetration) and still allow a large amount of blood to be treated, a microporous oxygenator consisting of multiple thin (0.5 to 1 cm) and wide (20 cm × 20 cm) modules might be developed. Application of phototherapy to all of the modules will require multiple LEDs to allow irradiation of the entire surface. We expect the irradiance (power of light per unit area) required to illuminate the surface of each module to be similar to the irradiance used in this study (80 mW/cm²). The total power required to illuminate a modular device with a 0.2- to 0.3-m² surface area of exposure will be about 150 to 250 W.

The institution of prehospital extracorporeal cardiopulmonary resuscitation (ECPR) for cardiac arrest has been reported (46), and a randomized clinical trial comparing hospital-based and prehospital ECPR for cardiac arrest is ongoing (NCT02527031). The results of our study suggest that a minimally invasive technique, using low venous blood flow for extracorporeal blood phototherapy, may be used to treat CO-poisoned patients in the prehospital setting, at the
location where the CO exposure occurred. In patients with CO poisoning and concomitant impaired gas exchange, especially those who may be unstable for transportation to a medical center that has a hyperbaric chamber, “full” extracorporeal support at high blood flow may be used to provide oxygenation and CO2 removal. The results of this study suggest that the addition of phototherapy will markedly improve the rate of CO removal.

One limitation of this study is that it was performed in rats, and survival experiments were limited to a 1-hour follow-up after the beginning of treatment. Further development of a larger device and testing in larger animals will be required before translating this technology to humans. Another limitation of this study is that we did not directly compare ECCOR-P to HBO therapy. We believe that ECCOR-P and HBO have different applications. HBO is often not readily available in the initial period after CO exposure; in addition it would not be indicated and might cause additional damage to patients with acute lung injury. HBO is also technically difficult to administer to patients who are intubated and/or critically ill. In contrast, early application of low flow rate veno-venous ECCOR-P might increase the rate of CO elimination in the earliest phases after CO poisoning and may represent a unique, acute treatment for patients with severe lung injury. During HBO therapy at 2.5 atm O2, the P02 in the arterial blood perfusing peripheral tissues can be as high as 1200 to 1300 mmHg. In contrast, during veno-venous ECCOR-P and breathing NBO, the partial pressure in the arterial blood never exceeded 500 mmHg. Therefore, we expect treatment with ECCOR-P to result in less oxygen toxicity than treatment with HBO. A third limitation to this study is that we did not study the effect of early treatment with ECCOR-P on the long-term neurologic sequelae after CO poisoning. Last, we did not compare ECCOR-P to other therapies for the treatment of CO poisoning such as hydroxocobalamin (47), hemoCD (48), and H64Q neuroglobin (49). Because the mechanism by which phototherapy increases the rate of CO elimination (photodissociation of CO from hemoglobin) is different from the mechanism of action of CO scavengers, it is conceivable that a combination of treatments will facilitate CO removal in CO-poisoned patients. Further studies will be needed to evaluate and compare the efficacy of these therapies, when used either alone or in combination.

In summary, the veno-venous extracorporeal removal of CO from blood at low flow rates using a newly developed CO photo-remover increased the rate of CO elimination in a rat model of CO poisoning and improved survival in animals with concomitant lung injury. The successful development of a larger CO photo-remover combined with a minimally invasive system for extracorporeal blood phototherapy might be beneficial as a supplemental therapeutic strategy for the treatment of CO-poisoned patients.

**METHODS**

**Study design**

The objective of this study was to investigate the efficacy of extracorporeal removal of CO from blood using phototherapy as a possible therapy for CO poisoning. We developed an in vitro model of blood circulation and a miniaturized oxygenator suitable for blood phototherapy, CO photodissociation, and CO removal. We tested the device in a nonlethal model of CO poisoning in rats and studied the effect of extracorporeal CO photo-removal on the CO elimination rate, tissue oxygenation, and lactate clearance. We also tested this newly developed technology in a lethal model of combined CO poisoning and oleic acid–induced lung injury and studied the effect on gas exchange, CO elimination, hemodynamics, and survival rate. All the in vitro experiments were repeated at least three times. All animal studies were performed using protocols approved by the Institutional Animal Care and Use Committee at our institution and in accordance with National Institutes of Health guidelines. Animals were randomized into the study based on body weight to ensure equal distribution across groups. Sample size was chosen on the basis of pilot experiments that ensured a power of 80% and a significance of 5%. Investigators were not blinded to experiments. All data are included (no outlier values were excluded).

**Development of an extracorporeal CO photo-remover**

We developed a membrane oxygenator with a configuration suitable for blood phototherapy and tested the device in an in vitro model of veno-venous extracorporeal blood circulation. The in vitro circuit for blood circulation consisted of an open reservoir (10-ml syringe), a roller pump (NE-9000-G), silicone tubing, and a membrane oxygenator. Blood entering and exiting the CO photo-remover was collected, whereas the CO photo-remover was perfused with deoxygenated blood and ventilated with 100% oxygen at 1 liter/min. For each pair of samples, the actual oxygen transfer and the maximum oxygen transfer were calculated as follows:

- Actual O2 transfer (ml/min per m2) = \( \frac{C_{\text{post}O2} (\text{ml/dl}) - C_{\text{pre}O2} (\text{ml/dl})}{\text{BF (ml/min per m2)}} \)
- Maximum O2 transfer (ml/min per m2) = \( \frac{C_{\text{max}O2} (\text{ml/dl}) - C_{\text{pre}O2} (\text{ml/dl})}{\text{BF (ml/min per m2)}} \)

where

\[
C_{\text{pre}O2} (\text{ml/dl}) = [\text{Hb (g/dl)}] \times [\text{Sat pre}O2 (\%) \times 1.36 (\text{ml/dl}/100) + \text{Ppre}O2 (\text{mmHg}) \times 0.003]
\]

\[
C_{\text{post}O2} (\text{ml/dl}) = [\text{Hb (g/dl)}] \times [\text{Sat post}O2 (\%) \times 1.36 (\text{ml/dl}/100) + \text{Ppost}O2 (\text{mmHg}) \times 0.003]
\]

\[
C_{\text{max}O2} (\text{ml/dl}) = [\text{Hb (g/dl)}] \times [100 - \text{COHb}(\%) - \text{MetHb}(\%)] \times 1.36 (\text{ml/dl}/100) + \frac{[760 - 47\text{P}_{\text{po2}}CO2] (\text{mmHg})}{0.003}
\]

\[\text{BF (ml/min per m}^2) = \text{blood flow (ml/min)/gas exchange surface area (m}^2)\]

In all equations, \( C \) refers to content of oxygen in blood, “pre” refers to blood entering the oxygenator, and “post” refers to blood exiting the oxygenator.

In some experiments, after exposing human blood to 2% CO balanced with nitrogen, the rate of CO elimination was assessed, while the CO photo-remover was “ventilated” with 100% oxygen at 1 liter/min, with or without green (523 nm), red (623 nm), or blue (460 nm) phototherapy.

**Phototherapy**

LEDs (Mouser Electronics), with a maximum power of 750 mW, were used to produce green (523 nm), red (623 nm), or blue (460 nm) light (fig. S3). During ECCOR with phototherapy, the CO photo-remover was irradiated using a total of eight LEDs, four on each side of the membrane lung. The irradiance of phototherapy (the light power over the surface area of exposure) was about 80 mW/cm². LEDs were attached to heat sinks, which were ventilated with small fans to dissipate the heat produced during phototherapy.

**Animals**

All animal experiments were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital (Boston, MA). We studied anesthetized and mechanically ventilated Sprague-Dawley...
rats weighing 400 to 500 g. Rats were anesthetized with 5% isoflurane in oxygen for 3 to 5 min in a plexiglass chamber. After a tracheostomy, rocuronium (1 mg/kg) was injected intraperitoneally to induce muscle relaxation and rats were mechanically ventilated (Inspira; Harvard Apparatus). Volume-controlled ventilation was provided at a respiratory rate of 40 breaths/min, a tidal volume of 10 ml/kg, and positive end expiratory pressure (PEEP) of 2 cmH_2O. Anesthesia was maintained with 1 to 2% isoflurane, and continuous muscle relaxation was provided with rocuronium (2 to 4 mg/kg per hour). Airway pressure was continuously monitored, as well as end tidal CO2 (ETCO2), which was measured with a capnometer (PhysioSuite, CapnoScan End-Tidal CO2 Monitor, Kent Scientific).

The right carotid artery was cannulated with a PE20 catheter for blood sampling and arterial blood pressure monitoring. A bolus of heparin (200 UI/kg) and subsequent continuous infusion at 100 UI/kg per hour were administered for blood anticoagulation. A custom-made four-hole, 16-gauge cannula was placed in the right femoral vein, and an 18-gauge cannula (Intracran Safety, B. Braun Medical Inc.) was placed in the right jugular vein. Fluid resuscitation was maintained infusing 0.9% saline at a rate of 8 to 12 ml/kg per hour.

**Acute lung injury**

To induce acute lung injury and mimic a situation of impaired gas exchange such as may occur after thermal and smoke inhalation injuries, we used a model of intravenous administration of oleic acid as previously described (50). Oleic acid (99% pure, Sigma-Aldrich Corp.) was diluted in a 1% solution of albumin and saline in a 1:5 volume ratio. To induce lung injury, five 80-μl doses of this suspension were injected in the jugular vein every 10 min for a total of 400 μl. Injection of multiple doses of oleic acid, as opposed to a single-dose injection, prevented sudden death due to massive pulmonary embolism. During these experiments, the PEEP was set at 4 cmH_2O at baseline and increased to 7 cmH_2O when lung injury developed. Airway pressure was monitored continuously. The end-inspiration plateau pressure (P_{plat}) was measured after performing a 2-s inspiratory pause at the beginning of the experiment and at the end of CO poisoning before initiation of treatment. The compliance of the respiratory system (C_{rs}) was calculated as the ratio between the tidal volume and the difference between P_{plat} and PEEP.

**Veno-venous extracorporeal blood circulation in rats**

The extracorporeal circuit was primed with 10 to 12 ml of anticoagulated whole blood, obtained from a donor rat, and 3 to 5 ml of 0.9% saline. Before starting the extracorporeal blood circulation, the priming solution was warmed up to 37°C using two heating lamps. The extracorporeal circulation was initiated at 10 ml/min blood flow and progressively increased to 30 to 50 ml/min. Blood was drained by gravity from the femoral vein to a reservoir (10 ml syringe), circulated through the CO photo-remover using a roller pump (NE-9000-G), and reinfected via the right jugular vein. Body temperature was continuously monitored with a rectal temperature probe (Traceable Digital Thermometer, VWR). To estimate tissue oxygenation, an optical PO2 probe (Oxylab, Oxford Optronix) was inserted in the venous reservoir collecting blood from the inferior vena cava, allowing venous PO2 and blood temperature monitoring. Two heating lamps and a heating pad were used to maintain blood and body temperature between 37°C and 38°C. Of note, in the experiments with combined lung injury and CO poisoning, the extracorporeal circulation was initiated after the poisoning period.

**Measurements and calculations**

About 100 μl of arterial blood was collected at different time points for measurement of pH, P_{CO2}, P_{O2}, hemoglobin concentration (Hb), the fraction of oxyhemoglobin and carboxyhemoglobin (O2Hb and COHb, respectively) (ABL800 FLEX, Radiometer), and lactate concentration (Lactate Plus, Nova Biomedical) both during poisoning and treatment.

For both in vitro and in vivo experiments, the rate of decrease of COHb concentration during treatment was determined by fitting the COHb percentage at three or four time points after the beginning of the treatment using a single exponential decay equation (y = e^{-kx}). The time needed to reduce the initial concentration of COHb by 50% (COHb-t_{1/2}) was calculated as COHb-t_{1/2} = ln (2)/k. During the treatment period, the concentration of CO exhaled from the animal and removed by the CO photo-remover was measured using two CO gas analyzers (MSA Altair PRO; MSA Safety Inc.). The quantity of CO eliminated from the lungs and the membrane oxygenator was then calculated as the product between the concentration of CO and the minute ventilation or the sweep gas flow, respectively.

**Statistical analysis**

Statistical analysis was performed using GraphPad 7.0. Data were analyzed using Student’s t test and a one-way analysis of variance (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 ± SD unless specified otherwise.

**SUPPLEMENTARY MATERIALS**

stm.sciencemag.org/cgi/content/full/11/513/eaau4217/DC1

Fig. S1. Development of the CO photo-remover.

Fig. S2. Light sources.

Fig. S3. Hemodynamics during CO poisoning and treatment by breathing air with or without ECCOR-P.

Data file S1. Raw data (provided as separate Excel file).

View/request a protocol for this paper from Bio-protocol.

**REFERENCES AND NOTES**

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Phototherapy and extracorporeal membrane oxygenation facilitate removal of carbon monoxide in rats
Luca Zazzeron, Anna Fischbach, Walfre Franco, William A. Farinelli, Fumito Ichinose, Donald B. Bloch, R. Rox Anderson and Warren M. Zapol

Sci Transl Med 11, eaau4217.
DOI: 10.1126/scitranslmed.aau4217

Shining the light on blood
Carbon monoxide (CO) is a colorless, odorless gas that can cause severe illness and death after inhalation. After entering the bloodstream, CO replaces oxygen on hemoglobin, reducing oxygenation to peripheral tissues. When CO poisoning is associated with lung injury, treatment with 100% oxygen might not be effective. Leveraging previous studies showing that visible light was able to dissociate hemoglobin from CO, now, Zazzeron et al. developed an extracorporeal membrane oxygenator for blood exposure to visible light. The use of extracorporeal removal of CO using phototherapy, increased CO removal compared to 100% oxygen inhalation in rat model of CO poisoning and increased survival when CO poisoning was associated with lung injury.