Effects of inhaled carbon monoxide on acute lung injury in mice

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Carbon monoxide (CO) has long been known in biology and medicine as a toxic compound, due to its ability to bind hemoglobin with a much higher affinity than oxygen (25). Despite this image as a deadly gas, recent evidence indicates that CO may in fact have a cytoprotective function at low doses. CO is endogenously produced during heme metabolism by the enzyme heme oxygenase, which is one of the major antioxidant cytoprotective enzymes within the body (17, 22). Several investigators have reported that exogenously administered CO by inhalation may exert anti-inflammatory effects, providing protection in various animal models of tissue injury. In particular, the lung appears ideally placed for such treatment, and inhaled CO at concentrations of 250–500 parts per million (ppm) has been shown to be beneficial in a number of lung injury models, including hyperoxic injury (23, 24), allergen-induced inflammation (5), ischemia-reperfusion injury (12), lung transplant rejection (29), and ventilator-induced lung injury (9). However, the effectiveness of CO treatment in attenuating lung injury is not a completely universal experimental finding (8), and as the majority of previous studies have investigated relatively subacute, slowly developing models of injury, it is unclear whether inhaled CO may be useful in attenuating more rapidly progressing pulmonary inflammation in ALI and ARDS.

We therefore investigated the potential anti-inflammatory effects of inhaled CO in three in vivo mouse models of ALI, each of which display different pathophysiology to encompass the heterogeneous nature of human ALI. Initially, ALI induced by either intratracheal or intravenous lipopolysaccharide (LPS) was studied, as models of ALI/ARDS induced by both direct (pulmonary etiology) and indirect (extrapulmonary etiology) processes. Although CO has been reported to attenuate cytokine responses and improve mortality in LPS-induced endotoxin shock in mice (18, 21), the possible impact of CO on LPS-induced ALI in vivo is unclear. In addition, we investigated the impact of CO inhalation using the intravenous oleic acid (OA) model of ALI, which produces a clinical picture similar to human ARDS, and has previously been used to investigate potential therapies for ALI/ARDS (28). We were however unable to demonstrate anti-inflammatory effects or beneficial impact on physiological and immunological signs of
ALI due to CO exposure in any of the models used. The current data therefore do not support the use of inhaled CO to modify the progression of ALI.

METHODS

Animal Preparation

All experimental protocols were reviewed and approved by the UK Home Office in accordance with the Animals (Scientific Procedures) Act 1986. UK. Male C57BL/6 mice (Charles River, Margate, UK) aged 9–13 wk (20–30 g) were used throughout. Mice were anesthetized by intraperitoneal injection of 2.5 ml/kg Hypnorm (Fentanyl 0.8 mg/kg, Fluanisone 25 mg/kg) and 2.5 ml/kg Midazolam (12.5 mg/kg) and then acutely instrumented as described previously (7, 34, 35). In brief, mice were mechanically ventilated via tracheotomy using a custom-made mouse ventilator-pulmonary function testing system (11, 34). The left carotid artery was cannulated for monitoring of arterial blood pressure (BP), blood-gas analysis, and infusion of fluids. The right jugular vein was also cannulated in some animals for administration of LPS or OA. Anesthesia was maintained by further administration of the anesthetic mix via a catheter placed in the intraperitoneal cavity. Throughout the experiment, rectal temperature was monitored and maintained within the normal range by use of a heating pad.

Experimental Design

All animals were ventilated with tidal volume (Vt) of 9–10 ml/kg, 2–2.5 cmH2O positive end-expiratory pressure, and respiratory rate of 120 breaths/min, with either 100% O2 or a premixed 98% O2, 2% N2, 2–2.5 cmH2O positive end-expiratory pressure, and respiratory rate of 120 breaths/min, with either 100% O2 or a premixed 98% O2, 2% N2, 500 ppm CO gas mixture (Carburos Metalicos, Barcelona, Spain). CO content of the inspired gas was confirmed with an electrochemical CO analyzer (Testo 315-2; Testo, Alton, UK). After 1-h pre-exposure to either O2 or O2+CO, animals were allocated to the following protocols.

**Intratracheal LPS group.** LPS (20 μg, Escherichia coli O111:B4; Sigma, Poole, UK) in 25 μl of sterile saline was instilled directly into the trachea through the endotracheal tube. Animals were then ventilated with the same inspired gases and ventilator settings as described above for a further 3 h, after which animals were killed by anesthetic overdose. Lung lavage was carried out with 750-μl aliquots of sterile saline as described previously (34) for analysis of protein and cytokine concentrations and for differential cytology using Diff-Quik-stained samples prepared by Cytospin (Shandon, Runcorn, UK). A blood sample was removed via cardiac puncture for determination of carboxyhemoglobin (COHb) levels using a CO oximeter (IL Synthehsis, Bensenville, IL). A blood sample was then taken for analysis of plasma TNF levels, and the volume was replaced with sterile saline.

**Intravenous OA group.** A modification of our previously described mouse model of OA-induced lung injury was used (31). OA (0.07 ml/kg, Sigma) was infused over 1 min via the jugular vein by a syringe infusion pump at a rate of 0.1 ml/h. Animals were then ventilated with the same inspired gases for a further 2 h. At the end of the procedure animals were killed, and the lungs were lavaged with 750 μl of saline for analysis of protein and cytokine levels and for differential cytology.

**Physiological Measurements**

In all animals, airway pressure, airway flow, and mean BP were monitored continually throughout the experiments. Blood gas analyses were performed at baseline and at the end of the protocols. Respiratory system compliance and resistance were measured by the end-inflation occlusion technique (11, 34). Sustained inflation of 30 cmH2O for 5 s was performed every 30 min throughout experiments to avoid atelectasis. No animals died during any of the protocols used.

**Protein and Cytokines in Lavage Fluid and Plasma**

The protein concentration in lung lavage fluid was determined by the Bradford method (4) using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hemel Hempstead, UK) with bovine serum albumin (Sigma) as a standard. Determination of TNF concentration in plasma and lavage fluid was carried out using a custom-made sandwich ELISA as described previously (34). Macrophage inflammatory protein-2 (MIP-2) levels in lavage fluid were determined using a commercially available sandwich ELISA (R&D Systems, Abingdon, UK) according to the manufacturer’s instructions.

**Pulmonary Neutrophil Sequestration and Activation**

To evaluate the intravenous LPS-induced inflammatory response in the lung vasculature, a sensitive flow cytometry method recently developed in our laboratory (7) was used. In brief, lung cell suspensions were prepared from the excised lungs of mice by mechanical disruption, and cells were analyzed by flow cytometry (FACScalibur, BD, Oxford, UK). For quantification of pulmonary sequestration of polymorphonuclear neutrophils (PMN), PMN were identified by positive staining for FITC-conjugated rat anti-mouse Ly-6G (Gr-1) antibody (BD Pharmingen, Oxford, UK) and side scatter properties, and the absolute PMN counts recovered per lung were measured using Perfect-count microspheres (Caltag Medsystems, Towcester, UK) according to the manufacturer’s instructions. For assessment of cellular activation, lung-sequestered PMN were analyzed for surface expression of the adhesion molecules L-selectin and Mac-1 (CD11b), by simultaneous staining with phycocerythrin-conjugated rat anti-mouse L-selectin antibody (BD Pharmingen, Oxford, UK) and side scatter properties, and the absolute PMN counts recovered per lung were measured using Perfect-count microspheres (Caltag Medsystems, Towcester, UK) according to the manufacturer’s instructions. For assessment of cellular activation, lung-sequestered PMN were analyzed for surface expression of the adhesion molecules L-selectin and Mac-1 (CD11b), by simultaneous staining with phycocerythrin-conjugated rat anti-mouse L-selectin antibody (BD Pharmingen, Oxford, UK) and side scatter properties, and the absolute PMN counts recovered per lung were measured using Perfect-count microspheres (Caltag Medsystems, Towcester, UK) according to the manufacturer’s instructions. For assessment of cellular activation, lung-sequestered PMN were analyzed for surface expression of the adhesion molecules L-selectin and Mac-1 (CD11b), by simultaneous staining with phycocerythrin-conjugated rat anti-mouse L-selectin antibody (BD Pharmingen, Oxford, UK) and side scatter properties, and the absolute PMN counts recovered per lung were measured using Perfect-count microspheres (Caltag Medsystems, Towcester, UK) according to the manufacturer’s instructions. For assessment of cellular activation, lung-sequestered PMN were analyzed for surface expression of the adhesion molecules L-selectin and Mac-1 (CD11b), by simultaneous staining with phycocerythrin-conjugated rat anti-mouse L-selectin antibody (BD Pharmingen, Oxford, UK) and side scatter properties, and the absolute PMN counts recovered per lung were measured using Perfect-count microspheres (Caltag Medsystems, Towcester, UK) according to the manufacturer’s instructions. For assessment of cellular activation, lung-sequestered PMN were analyzed for surface expression of the adhesion molecules L-selectin and Mac-1 (CD11b), by simultaneous staining with phycocerythrin-conjugated rat anti-mouse L-selectin antibody (BD Pharmingen, Oxford, UK) and side scatter properties, and the absolute PMN counts recovered per lung were measured using Perfect-count microspheres (Caltag Medsystems, Towcester, UK) according to the manufacturer’s instructions.

**Statistics**

The data are expressed as mean values ± SD. Changes in parameters across the course of the experiments and differences between groups were analyzed by one-way ANOVA with Scheffé’s tests or Student’s t-tests. A P value of <0.05 was considered significant.

RESULTS

**Intratracheal LPS Exposure**

Physiological measurements and blood gas. To confirm the efficacy of CO exposure, blood COHb levels were determined at the end of the intratracheal LPS protocol. The levels of COHb were undetectable (0% of total hemoglobin) in all animals exposed to O2 alone (n = 5) and 10.7 ± 1.4% of total hemoglobin in CO-treated animals (n = 5). Intratracheal instillation of 20 μg of LPS produced an initial increase in peak inspiratory pressure (PIP) in all animals due to the presence of fluid within the airways (Table 1). Over the course of the experiments, PIP returned toward preinstillation values, indicating clearance of intratracheal fluid, rather than any LPS-induced deterioration in respiratory mechanics. Arterial BP and
Blood gases were well maintained within normal limits throughout the procedures. Inhalation of 500 ppm CO had no significant impact on these physiological variables.

Neutrophil infiltration. Instillation of LPS into the lungs produced a significant recruitment of neutrophils into the alveolar space compared with untreated control animals (Fig. 1). Exposure of animals to CO had no impact on neutrophil recruitment, when expressed either as % neutrophils or total neutrophil number recovered in lavage fluid.

Protein and cytokine analysis. Intratracheal LPS produced an increase in lavage fluid total protein concentration compared with untreated animals, indicating the development of pulmonary edema (Fig. 2A). Lavage fluid levels of the cytokines TNF and MIP-2, a murine functional homolog of the human neutrophil chemoattractant IL-8, were dramatically increased by intratracheal LPS administration (Fig. 2, B and C). CO had no effect on the levels of protein or proinflammatory cytokines in lung lavage fluid. TNF was undetectable (<7 pg/ml) in plasma in animals treated with intratracheal LPS.

Intravenous LPS Exposure

Physiological measurements and blood gas. Intravenous administration of 20 μg of LPS did not affect PIP but did cause a progressive decrease in arterial BP, indicating endotoxin-induced hypotension (Table 2). Blood gases remained within relatively physiological limits throughout, although pH declined over time, consistent with progressive deterioration in organ perfusion with intravenous LPS. Overall there was little sign that physiological variables were affected by CO exposure, although PO2 was found to be lower in the CO group.

### Table 1. Physiological parameters in animals administered intratracheal LPS

<table>
<thead>
<tr>
<th></th>
<th>Pre-LPS</th>
<th>LPS + 60 min</th>
<th>LPS + 120 min</th>
<th>LPS + 180 min</th>
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<tr>
<td><strong>LPS it</strong></td>
<td></td>
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<tr>
<td>PIP, cmH2O</td>
<td>13.1 ± 2.4</td>
<td>16.0 ± 2.3*</td>
<td>14.8 ± 2.3*†</td>
<td>14.6 ± 2.3†</td>
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<tr>
<td>BP, mmHg</td>
<td>56 ± 9</td>
<td>63 ± 8</td>
<td>64 ± 9†</td>
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<td>pH</td>
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<td>PCO2, mmHg</td>
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<td>PO2, mmHg</td>
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<tr>
<td>PIP, cmH2O</td>
<td>14.5 ± 1.1</td>
<td>16.2 ± 2.3†</td>
<td>15.8 ± 2.0</td>
<td>15.0 ± 1.6</td>
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<tr>
<td>BP, mmHg</td>
<td>68 ± 6</td>
<td>64 ± 9</td>
<td>64 ± 6</td>
<td>63 ± 6</td>
</tr>
<tr>
<td>pH</td>
<td>7.44 ± 0.02</td>
<td>7.38 ± 0.04*</td>
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<td>PCO2, mmHg</td>
<td>39.9 ± 4.1</td>
<td>38.4 ± 6.4</td>
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<td></td>
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<tr>
<td>PO2, mmHg</td>
<td>332 ± 20</td>
<td>337 ± 52</td>
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</table>

Values are means ± SD; n = 5 for all observations. Measurements were taken before LPS administration, and every 60 min thereafter. BP was well maintained within a reasonable range expected for C57BL/6 mice anesthetized with Hypnorm and Midazolam (38). PIP, peak inspiratory pressure; BP, mean arterial blood pressure. *P < 0.01; †P < 0.05; vs. pre-LPS; ‡P < 0.05 vs. LPS it group.

Fig. 1. Recovery of neutrophils (PMN) in lavage fluid from animals administered intratracheal (it) LPS and ventilated using either O2 or O2 + 500 ppm CO and in untreated control animals. Results are expressed as either percentage (A) or total number (B) of PMN recovered. *P < 0.01 vs. untreated controls; n = 5 per group.

Fig. 2. Total protein (A) and cytokines TNF (B) and macrophage inflammatory protein (MIP)-2 (C) in lavage fluid from animals administered intratracheal LPS and ventilated using either O2 or O2 + 500 ppm CO and in untreated control animals. *P < 0.01 vs. untreated controls; n = 5 per group.
Table 2. Physiological parameters in animals administered intravenous LPS

<table>
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<th>Pre-LPS</th>
<th>LPS + 60 min</th>
<th>LPS + 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIP, cmH2O</td>
<td>10.2 ± 1.2</td>
<td>11.0 ± 1.4†</td>
<td>10.8 ± 1.3</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>63 ± 11</td>
<td>51 ± 5</td>
<td>44 ± 4†</td>
</tr>
<tr>
<td>pH</td>
<td>7.44 ± 0.07</td>
<td>7.35 ± 0.04*</td>
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<tr>
<td>Pco2, mmHg</td>
<td>40.2 ± 8.4</td>
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<tr>
<td>Po2, mmHg</td>
<td>344 ± 32</td>
<td>342 ± 24</td>
<td></td>
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</tbody>
</table>

Values are means ± SD; n = 5 for all observations. Measurements were taken before LPS administration, and every 60 min thereafter. *P < 0.01, †P < 0.05, vs. pre-LPS; ‡P < 0.05 vs. LPS iv group.

Neutrophil sequestration and activation. Intravenous LPS produced negligible neutrophil migration into the alveolar space in all animals. However, flow cytometric analysis of lung cell suspension demonstrated that intravenous LPS infusion produced a dramatic increase in the number of lung-sequestered PMN compared with untreated controls (Fig. 3A). These lung–sequestered PMN were also found to be activated by LPS in terms of increased Mac-1 expression and decreased L-selectin expression, indicating increased L-selectin shedding (Fig. 3, B and C). Both lung PMN sequestration and activation were unaffected by CO treatment.

Cytokine analysis. TNF levels in plasma 1 h after intravenous LPS infusion were greatly increased compared with the undetectable levels observed in untreated controls and intratracheal LPS animals but were not affected by CO exposure (1,040 ± 350 pg/ml for O2 animals vs. 1,060 ± 350 pg/ml for O2+CO animals, n = 5 for each).

Intravenous OA

Physiological measurements and blood gas. Intravenous OA administration provoked a significant increase in PIP in all animals (Table 3), which was associated with a decline in respiratory system compliance (47 ± 10% decrease for O2 animals vs. 52 ± 2% decrease for O2+CO animals compared with pre-OA values, n = 5 for each). OA also caused a significant decrease in arterial BP and deterioration of gas exchange, i.e., decreased pH, increased Pco2, and decreased Po2 (Table 3). However, these physiological signs of ALI were not significantly different between animals exposed to O2 alone or O2 with CO (except that Po2 was lower in CO group at baseline).

Neutrophil infiltration. Intravenous OA administration induced a dramatic neutrophil infiltration into lavage fluid (Fig. 4), which was not attenuated by CO exposure.

Protein and cytokine analysis. Total protein content in lavage fluid was dramatically increased in all OA-treated animals compared with untreated mice, consistent with the development of pulmonary edema, but this was not affected by CO exposure (4.8 ± 0.4 for O2 animals vs. 4.7 ± 0.9 mg/ml for O2+CO animals, n = 5 for each). Lavage fluid concentrations of both TNF (5.7 ± 10.8 for O2 animals vs. 14.1 ± 8.4 pg/ml for O2+CO animals, n = 5 for each) and MIP-2 (4.4 ± 1.1 for O2 animals vs. 3.8 ± 0.6 pg/ml for O2+CO animals, n = 5 for each) were very low in all OA-treated animals and unaffected by the presence of CO.

Discussion

In the current study we investigated the putative anti-inflammatory effects of inhaled CO on three models of ALI of different pathophysilogies in mice. We studied both inflammatory (neutrophil infiltration and cytokine levels in lavage fluid, pulmonary neutrophil sequestration and activation) and physiological (respiratory mechanics, blood gas variables) markers of ALI in each of these three models. We were, however, unable to determine a positive effect of CO exposure on the measured parameters in any of the models used.

In recent years it has become apparent that activity of the enzyme heme oxygenase-1 (HO-1) confers protection to tissues against oxidative injury. HO-1 has been demonstrated to possess anti-inflammatory, antiapoptotic, and antiproliferative effects (17), although the mechanisms behind this are unclear.
CO provides cytoprotection is considered to be the attenuation of physiological lung injury developed, including deteriorating respiratory mechanics and abnormalities in gas exchange, as well as a large influx of neutrophils into the alveolar space. In the presence of 500 ppm CO, however, neither physiological lung injury markers nor the consequent pulmonary inflammation was attenuated.

Table 3. Physiological parameters in animals administered intravenous OA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-OA</th>
<th>60 min post-OA</th>
<th>120 min post-OA</th>
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<tr>
<td>OA</td>
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<tr>
<td>PIP, cmH2O</td>
<td>10.1 ± 0.4</td>
<td>14.7 ± 1.5*</td>
<td>16.4 ± 2.0*</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>62 ± 2*</td>
<td>51 ± 3*</td>
<td>50 ± 3*</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.02</td>
<td>7.24 ± 0.03*</td>
<td></td>
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<tr>
<td>PO2, mmHg</td>
<td>40.3 ± 3.0</td>
<td>63.7 ± 4.2*</td>
<td></td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>379 ± 23</td>
<td>133 ± 33*</td>
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<tr>
<td>OA + CO</td>
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<tr>
<td>PIP, cmH2O</td>
<td>10.4 ± 0.3</td>
<td>15.2 ± 1.8*</td>
<td>17.7 ± 1.5*</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>58 ± 5*</td>
<td>48 ± 4*</td>
<td>47 ± 4*</td>
</tr>
<tr>
<td>pH</td>
<td>7.45 ± 0.02</td>
<td>7.24 ± 0.04*</td>
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<tr>
<td>PO2, mmHg</td>
<td>37 ± 3.9</td>
<td>50.8 ± 14.0*</td>
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</tr>
<tr>
<td>PO2, mmHg</td>
<td>329 ± 32*</td>
<td>109 ± 31*</td>
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</table>

Values are means ± SD; n = 5 for all observations. Measurements were taken before OA administration, and every 60 min thereafter. OA, oleic acid. *P < 0.01, †P < 0.05, vs. pre-OA; ‡P < 0.05 vs. OA group.

Cytoprotection may be provided by the byproducts of HO-1 catalysis of heme protein, i.e., biliverdin, iron, and CO (22). CO in particular has been the subject of intense research, as it is a stable molecule, and being gaseous it is easily administered by inhalation. Beneficial effects of low-dose CO inhalation have been shown in various models of organ injury, both in the lung (5, 9, 12, 23, 24, 29) and in distal organs such as small intestine (16) and liver (37). The major mechanism by which CO provides cytoprotection is considered to be the attenuation of inflammatory responses (20), with CO exposure reported to reduce LPS-induced production of proinflammatory cytokines including TNF, IL-6, IL-1β, granulocyte/macrophage colony-stimulating factor, and MIP-1β, both in vivo in mice (18, 21) and in isolated macrophages (18, 21, 27). Inhaled CO therapy may therefore be of great use in the intensive care unit, particularly in the treatment of ALI and ARDS. These syndromes are a major cause of mortality with substantial inflammatory components and, as lung disorders, would seem to be particularly amenable to inhaled gas therapy.

To investigate the possible beneficial effects of inhaled CO on ALI, we initially used in vivo mouse models of endotoxin-induced ALI. As ALI/ARDS is induced by both pulmonary and nonpulmonary etiologies, we studied the response to intratracheal and intravenous LPS administration. Both models produced substantial pulmonary inflammation, although the pathophysiology involved was different, with the inflammatory response being relatively compartmentalized in either the alveolar or vascular space (13, 14, 19). Intratracheal LPS induced lung injury with clear alveolar inflammation, characterized by intra-alveolar neutrophil infiltration and increased lavage fluid proinflammatory cytokines (TNF and MIP-2). There was also an increase in protein concentration in lavage fluid. Intravenous LPS induced lung injury of a predominantly vascular nature. Animals injected with intravenous LPS did not show increases in lavage fluid neutrophils within the time frame of this study, consistent with previous demonstrations that transvascular migration of neutrophils and increases in alveolar space cytokines requires more time and higher doses of intravenous LPS (3). On the other hand, there was an increase in plasma TNF and significant activation and sequestration of neutrophils within the lung vasculature, as assessed by flow cytometry. In both models, however, we did not observe any in vivo effect of CO on these immunological parameters of neutrophil recruitment and cytokine activation. This may be particularly significant in the case of intratracheal LPS administration, as alveolar macrophages, which are considered to play a major role in producing inflammatory cytokines and triggering neutrophil infiltration in this model (36), would have been directly exposed to inhaled CO.

Because there were few physiological signs of lung injury in the above LPS models, we also investigated the impact of CO exposure on OA-induced ALI. This model produces a clinical picture very similar to human ARDS and has been frequently used to study potential treatments for ALI/ARDS (28). The precise mechanisms by which OA promotes lung injury are not clear but appear to involve the formation of microemboli and production of reactive oxygen species leading to endothelial and epithelial cell damage (28), induction of apoptosis (15), and fibrin deposition (28), culminating in a rapid pulmonary inflammatory response. It has been suggested that CO enhances fibrinolysis via cGMP signaling in ischemic lung injury (12), possesses antiapoptotic properties in lung transplant injury (29), and provides protection against oxidative stress (23). We therefore reasoned that CO may be protective against OA-induced lung injury. After OA infusion, clear signs of physiological lung injury developed, including deteriorating respiratory mechanics and abnormalities in gas exchange, as well as a large influx of neutrophils into the alveolar space. In the presence of 500 ppm CO, however, neither physiological lung injury markers nor the consequent pulmonary inflammation was attenuated.

![Fig. 4. Recovery of PMN in lavage fluid from animals administered intravenous oleic acid (OA) and ventilated using either O2 or O2 + 500 ppm CO. Results are expressed as either percentage (A) or total number (B) of PMN recovered. *P < 0.01 vs. OA group; n = 5 per group.](http://ajplung.physiology.org/)

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These results are clearly in contrast to a number of previous studies that have demonstrated beneficial effects of CO on lung injury (5, 9, 12, 23, 24, 29). This is, however, not the only study that has failed to show a positive impact of CO in vivo. Clayton et al. (8) found that inhaled CO did not significantly attenuate lung injury induced by hyperoxia in a rat model very similar to the one Otterbein et al. (23) used to show a positive effect. Clayton et al. (8) pointed out a lack of concordance of blood COHb values with gas equilibrium calculations in Otterbein’s study, in which the COHb levels were unexpectedly high (6.6%) at baseline without CO and increased to 11.3% at only 250 ppm CO. They suggested that the CO measurement in Otterbein’s study may not be accurate and that the observed protecting effects of CO in hyperoxic lung injury could be due to higher doses of CO or lower inspired oxygen (8). In the current study we delivered CO by a ventilator into the animal lungs in the form of a premixed 500 ppm CO/O2 gas mixture, precluding any potential problem in the inspired CO dose, and found blood COHb levels of ~11%, which is comparable with previously reported values of COHb (10–14% at CO 200–500 ppm) delivered in 95–98% oxygen (8, 23, 24). It is possible that higher blood COHb levels, if achieved either by the use of gas mixtures containing air as opposed to 98% O2 or by the inhalation of higher concentrations of CO, could have led to positive results. However, such treatment protocols do not have much clinical relevance, because the ventilatory management of ALI/ARDS usually requires high inspired O2 levels, and inhaled CO >500 ppm might be associated with apoptosis in the brain (8). Moreover, previous dose-response studies of anti-inflammatory effects of inhaled CO have indicated positive effects at doses as low as 10–50 ppm (9, 21, 23). Taken together, it appears unlikely that the current negative results are related to insufficient CO dose or CO delivery to the animals.

Other explanations for the lack of a positive effect of inhaled CO include the possibility that the models used in our study were too severe to show small to moderate effects of inhaled CO, or that the techniques used to assess inflammation were insufficiently sensitive to detect changes induced by CO. However, we believe it unlikely that these explanations could apply to all of the three models used in the current study. In particular, using similar mouse models of ALI induced by intratracheal LPS, we (7) and others (26) have shown that intravenous CD18 antibody markedly attenuated neutrophil infiltration into the alveolar space. The dose of intravenous LPS used in the current study (0.7–1 mg/kg) is comparable to the intraperitoneal LPS doses used in previous studies demonstrating benefits of CO in mice (18, 21). Dolinay et al. (9) recently reported in rats that CO exposure did not affect small to moderate lung inflammation induced by either LPS (3 mg/kg iv) or high VT mechanical ventilation but did attenuate more severe injury induced by the combined inflammatory (LPS) and mechanical (ventilatory) insults, although interpretation of such data is complicated because multi-insult lung injury models may potentially produce unexpected and model-specific interactions (10, 33). In addition, many of the techniques used (cytokine ELISA, neutrophil accumulation in lavage fluid) have been used previously to show CO-induced organ protection, and we have demonstrated that the flow cytometry-based method used in the intravenous LPS model is able to detect small differences in pulmonary neutrophil sequestration between different mechanical ventilatory strategies (7).

Finally, it is possible that CO exposure may have prolonged effects on ALI that could not be detected in the current study, due to the acute (2–3 h) nature of the injury models used. Even if inhaled CO is not effective in reversing or modifying acute overwhelming inflammation in the lung as seen in the ALI models in our study, more prolonged exposure to CO may have some beneficial effects in attenuating subacute to chronic lung inflammation or facilitating the resolution from lung injury. In clinical ALI/ARDS, the disease process develops more slowly than that in animal models, and hence prolonged exposure to inhaled CO may change the course of ALI, leading to better recovery and survival following the acute episode. This possibility was not excluded by the results of the current study and remains to be further investigated.

In summary, using three different in vivo mouse models of ALI, the current study did not find evidence to support the hypothesis that inhaled CO therapy is beneficial in the treatment of ALI and ARDS. Considering the potential side effects of CO, our results suggest that further studies are needed to clarify any potential role of CO in modifying the progression of ALI.

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DISCLOSURES

M. Takata has worked as a consultant to Carburos Metalicos.

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