Review
Bench-to-bedside review: Carbon monoxide - from mitochondrial poisoning to therapeutic use

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Abstract

Carbon monoxide (CO) is generated during incomplete combustion of carbon-containing compounds and leads to acute and chronic toxicity in animals and humans depending on the concentration and exposure time. In addition to exogenous sources, CO is also produced endogenously by the activity of heme oxygenases (HOs) and the physiological significance of HO-derived CO has only recently emerged. CO exerts vasoactive, anti-proliferative, anti-oxidant, anti-inflammatory and anti-apoptotic effects and contributes substantially to the important role of the inducible isoform HO-1 as a mediator of tissue protection and host defense. Exogenous application of low doses of gaseous CO might provide a powerful tool to protect organs and tissues under various stress conditions. Experimental evidence strongly suggests a beneficial effect under pathophysiological conditions such as organ transplantation, ischemia/reperfusion, inflammation, sepsis, or shock states. The cellular and molecular mechanisms mediating CO effects are only partially characterized. So far, only a few studies in humans are available, which, however, do not support the promising results observed in experimental studies. The protective effects of exogenous CO may strongly depend on the pathological condition, the mode, time point and duration of application, the administered concentration, and on the target tissue and cell. Differences in bioavailability of endogenous CO production and exogenous CO supplementation might also provide an explanation for the lack of protective effects observed in some experimental and clinical studies. Further randomized, controlled clinical studies are needed to clarify whether exogenous application of CO may turn into a safe and effective preventive and therapeutic strategy to treat pathophysiological conditions associated with inflammatory or oxidative stress.

Carbon monoxide: exogenous sources and toxic effects

High concentrations of carbon monoxide (CO) are generated during incomplete combustion of carbon-containing compounds such as wood, coal, gas, oil, or tobacco. CO is a colorless and odorless gas that causes acute and chronic toxicity in humans and animals. CO mediates its toxic effects primarily by strongly binding to hemoglobin and forming carboxyhemoglobin (COHb), thereby reducing the oxygen-carrying capacity of the blood. The affinity of hemoglobin for CO is approximately 210 to 250 times that for oxygen [1]. Both decreased arterial oxygen content (impaired O_2 binding to hemoglobin) and decreased tissue oxygen pressure (PO_2; increased affinity of COHb for O_2) lead to tissue hypoxia [2,3]. There is a linear correlation between the inspired level of CO and arterial COHb levels [4]. Although the percentage of COHb in blood represents the best predictive marker for extrapolating the total amount of CO, COHb levels do not always correlate with the degree of injury and outcome [5]. COHb levels between 15 and 20% seem to be well tolerated in humans and are considered the 'biological threshold' above which severe CO-mediated injury is likely to occur [6]. In addition to hemoglobin, CO binding to other heme-containing proteins, such as cytochrome c oxidase (thus interfering with cellular respiration), catalase, or myoglobin, may partly contribute to the toxic effects.

The most vulnerable organs to CO-induced hypoxia are the heart and the brain because of their high metabolic rate [7]. The mild symptoms of acute CO poisoning are often non-specific and include headache, nausea, vomiting, dizziness, and fatigue, which may progress to confusion, tachypnea, tachycardia, impaired vision and hearing, convulsions, loss of consciousness, finally leading to death when immediate and adequate treatment is not available. The amount of CO inhaled and/or the exposure time are the most critical factors that determine the severity of CO poisoning. In addition, children and older adults are more susceptible and may have more severe symptoms [8]. Predisposing conditions for CO toxicity have been described, such as cardiovascular disorders (for example, coronary heart disease), chronic obstructive pulmonary disease (COPD), or anemia [9]. Heavy

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CO = carbon monoxide; COHb = carboxyhemoglobin; COPD = chronic obstructive pulmonary disease; CO-RM = carbon monoxide-releasing molecule; HO = heme oxygenase; IL = interleukin; LPS = lipopolysaccharide; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor-κB; sGC = soluble guanylate cyclase; TNF = tumor necrosis factor.

smokers may have more severe symptoms since their COHb levels are already elevated.

Carbon monoxide appears to be the leading cause of injury and death due to poisoning worldwide [10]. Since tissue hypoxia is the underlying mechanism of CO-induced injury, increasing the inspired oxygen concentration represents the treatment for CO poisoning. In severe poisoning, hyperbaric oxygen therapy is regarded as the therapy of choice [11]. Both normobaric and hyperbaric oxygen improve oxygen delivery by increasing the amount of oxygen dissolved in plasma and by reducing the half-life of COHb. However, the results from existing randomized, controlled trials of hyperbaric versus normobaric oxygen in the treatment of acute CO poisoning provide conflicting results regarding the effectiveness of hyperbaric oxygen for the prevention of neurological symptoms [12]. An ongoing phase IV randomized clinical trial investigates important clinical outcomes (for example, 6-week cognitive sequelae) of patients with acute CO poisoning randomized to receive either one or three hyperbaric oxygen treatments [13]. The estimated study completion date is May 2009. If treatment of CO poisoning is timely, most patients are able to recover, but even with adequate treatment CO poisoning may result in permanent memory loss or brain damage. For the long-term sequelae of acute CO poisoning, only symptomatic therapy is available. Chronic exposure to CO may lead to myocardial hypertrophy [14].

Functions of endogenous carbon monoxide production

Coburn and colleagues [15] demonstrated that CO is endogenously produced in animals and humans. The vast majority of endogenous CO is derived from the oxidative breakdown of heme by microsomal heme oxygenases (HOs). HO catalyzes the first and rate-limiting step in heme degradation, yielding equimolar amounts of CO, iron, and biliverdin-IXα (Figure 1), which is further converted to bilirubin by biliverdin reductase [16]. Two isoforms of HO have been described, namely HO-1 [17,18] and HO-2 [19,20]. Furthermore, a third isoform has been found in rats [21], which represents a processed pseudogene derived from the gene for HO-2 [22]. HO-2 is constitutively expressed in many tissues, with high activity in testes, central nervous system, liver, kidney, and intestine. A basal expression of HO-1 is found in tissues that degrade senescent red blood cells, predominantly spleen, reticuloendothelial cells of the liver and bone marrow [23]. HO-1 is the inducible isoform, and induction of HO-1 gene expression occurs in response to a wide variety of endogenous and exogenous stimuli, such as chemical or physical stimuli, xenobiotics, hyperoxia, hypoxia, ischemia/reperfusion, inflammation, surgical procedures, or anesthetics [24-29].

The critical role of HO-1 under physiological conditions was demonstrated in the first described case of human HO-1 deficiency. The boy in this case presented with severe growth

retardation, persistent hemolytic anemia, and severe, persistent endothelial damage [30] and died at the age of 6 years [31]. Over the past decade the function of HO-1 has expanded from a heme-degrading enzyme to a key mediator of tissue protection and host defense, and its cytoprotective effects have been described *in vivo* and *in vitro* [24,25,28,32-42].

The products of the HO pathway - CO, iron, and biliverdin/bilirubin - have long been regarded solely as waste products. Recently, the unique biological functions of the products and their contribution to the protective effects of the HO system have attracted great interest. Thus, the HO system has different functions: besides the breakdown of heme, a pro-oxidant [43], it produces cytoprotective substances, and the inducibility of HO-1 renders it a powerful endogenous cytoprotective system.

Bilirubin has been described as a potent endogenous anti-oxidant [44] with potential clinical implications [45]. Free iron exhibits oxidizing capacities, although the iron released during heme degradation stimulates the synthesis of ferritin [46], which sequesters unbound iron, thereby serving as an additional anti-oxidant [47]. The observation that CO can weakly activate soluble guanylate cyclase (sGC), thereby stimulating the production of cGMP, suggested an important role of CO as an intracellular messenger molecule, thus acting in a similar way to nitric oxide [48,49]. The functions of CO as a neural messenger have since been described [50]. Vasoactive effects of CO have been reported in the pulmonary vasculature [51] and in the liver [37,52], where CO acts to maintain portal venous vascular tone in a relaxed state [37]. In addition to the biological functions of CO under physiological conditions, the substantial contribution of CO to the protective effects of induced HO activity has recently been recognized and includes vasoactive, anti-oxidative, anti-inflammatory, anti-apoptotic, and anti-proliferative properties. Thus, CO has advanced from a toxic waste product to a physiological regulator and the importance of endogenously derived CO to control homeostasis under both physiological and pathophysiological conditions is increasingly recognized in every organ system and cell type.

Although different mechanisms explaining the effects of CO have been described, the exact underlying signaling mechanisms and precise molecular targets of CO are only partially elucidated. Effects mediated by CO-induced activation of sGC/cGMP include inhibition of platelet activation and aggregation, smooth muscle relaxation, vasoactive effects, inhibition of cellular proliferation, and effects on neurotransmission [37,49-56]. cGMP-independent mechanisms of vasoregulation have also been suggested. CO may directly activate calcium-dependent potassium channels, thus mediating dilation of blood vessels [57]. Recent evidence suggests an important role of CO as a signaling molecule in modulating mitogen-activated protein kinases (MAPKs),

Figure 1
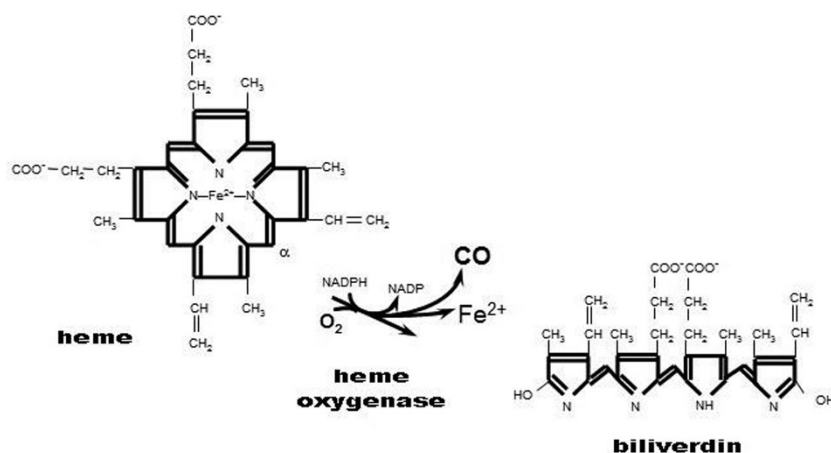
Heme oxygenase pathway. Heme oxygenase catalyzes the rate-limiting step in the degradation of heme leading to the generation of equimolar amounts of free iron, biliverdin and carbon monoxide.

especially p38 MAPK in response to oxidative stress and inflammation (reviewed in [58,59]). CO-mediated activation of p38 MAPK has been shown to exert anti-inflammatory [60], anti-apoptotic, and anti-proliferative effects [61,62]. Downstream target molecules of CO-dependent p38 MAPK activation have been identified, namely heat shock protein 70 and caveolin-1 [61,62]. Zhang and colleagues [63] demonstrated that the anti-apoptotic effects of CO involve both phosphatidylinositol 3-kinase/Akt and p38 MAPK signaling pathways in endothelial cells in a model of anoxia-reoxygenation injury. In hepatocytes, CO activated nuclear factor-κB (NF-κB) through a mechanism that involves reactive oxygen species-induced Akt phosphorylation and protected against cell death [64]. Figure 2 provides a simplified overview of the described CO-mediated signal transduction pathways.

Therapeutic applications of carbon monoxide

The observation that induction of HO-1 gene expression under pathological conditions plays an important role in organ preservation strongly suggests that CO might be substantially involved in mediating these effects. This is supported by the observation in models of HO-1 deficiency or after blockade of HO activity that the protective effects of induction of HO-1 are mimicked by low amounts of exogenous CO [54,59,65]. However, pre-induction of the HO-1 system by exogenous stimuli to induce local CO release or exogenous application of CO to potentiate the endogenous protective effects may be challenging. To increase the availability of CO, different approaches have been developed, including induction of HO-1 gene expression with pharmacological and genetic strategies, inhalation of low doses of CO, and application of CO-releasing molecules. Figure 3 briefly summarizes the protective effects and the potential therapeutic applications of CO in a variety of disorders and diseases of different organ systems.

### Induction of HO-1 gene expression

Strategies to induce HO-1 as a protective mechanism against a subsequent stress event include pharmacological approaches such as volatile anesthetics [40] or heme derivatives [32,33], and genetic approaches [39] as well as the use of other inducers as described above. Long-term overexpression of HO-1 by targeted gene transfer has become a powerful tool to investigate the specific role of the HO-1 enzyme [66]. The amount of CO released by the induced activity of HO-1 is unknown. In addition, induction of HO-1 increases the concentration of all products of the pathway, and the contribution of CO to the observed protective effects is difficult to evaluate.

Exogenous application of carbon monoxide

Inhalation of CO represents a novel therapeutic approach and exerts both local effects on the lungs and systemic effects. The challenge remains to reach safe and effective concentrations in target tissues without producing deleterious effects caused by CO-mediated tissue hypoxia. The tolerance to CO exposure has been investigated in rodents and conflicting results have been obtained: while continuous application of 500 ppm CO for 2 years had no deleterious effects [67], 200 ppm for 20 h per day over 14 days induced myocardial hypertrophy [14].

The CO-releasing properties of transition metal carbonyls were first described by Herrman [68]. Motterlini and his group have developed CO-releasing molecules (CO-RMs) as a new strategy to deliver defined amounts of CO for therapeutic applications [6,69] without significantly affecting COHb levels [70]. In particular, the synthesis of a water-soluble compound might be promising. So far, only experimental data are available. The use of CO-RMs to characterize CO-mediated cytoprotection has been reviewed by Foresti and colleagues [6].

Figure 2
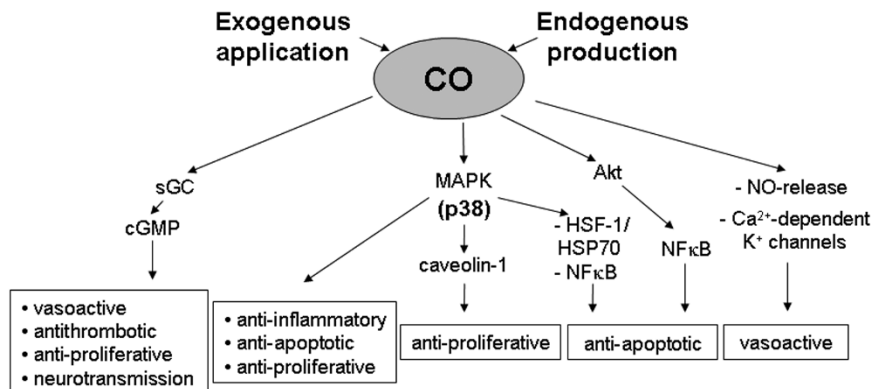
Carbon monoxide signal transduction pathways. CO, carbon monoxide; HSF, heat shock factor; HSP, heat shock protein; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor-κB; NO, nitric oxide; sGC, soluble guanylate cyclase.

Figure 3
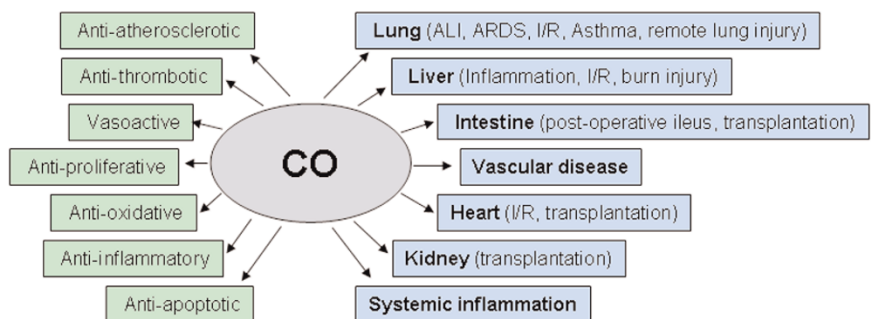
Protective effects and potential therapeutic applications of carbon monoxide. ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CO, carbon monoxide; I/R, ischemia/reperfusion.

Preclinical experimental studies
In most experimental models, acute rather than chronic inhalation of CO is applied (10 to 1,000 ppm for 1 to 24 h). Depending on the concentration, different exposure times are required to reach COHb equilibrium [71]. CO inhalation has been shown to be protective in experimental inflammatory and non-inflammatory disease models (reviewed in [6,25, 72-75]). The majority of studies investigating the effects of low amounts of inhaled CO concentrate on disease models in the lungs. In addition to local effects in the lungs, inhaled CO is also able to affect systemic organ dysfunction.

Lung The protective effects of inhaled CO have been investigated in models of acute lung injury, acute respiratory distress syndrome (ARDS), ischemia/reperfusion, asthma, and remote lung injury. The first *in vivo* evidence to suggest a therapeutic potential of low dose gaseous CO was provided by Otterbein and colleagues [76]. Rats exposed to low concentrations of CO exhibited a significant attenuation of hyperoxia-induced lung injury and increased survival. CO

exposure exerted anti-inflammatory and anti-apoptotic effects. The molecular mechanisms of the observed inhibition of pro-inflammatory cytokines involve the MKK3/p38 MAPK pathway [77]. In contrast, low levels of CO were not protective in a similar rat model of hyperoxic acute lung injury [4]. Inhalation of CO attenuated the development of hypoxia-induced pulmonary artery hypertension in rats, presumably through activation of Ca^{2+}-activated K^+ channels [78] and was also able to reverse established pulmonary hypertension [79]. Inhalation of CO for 6 h after intratracheal injection of acidic solution in mice reduced early neutrophil recruitment without affecting chemokine levels in bronchoalveolar fluid [80]. The pathomechanisms of allergen-induced asthma include inflammation and bronchoconstriction. In ovalbumin-induced asthma, CO treatment of mice for 2 h before aerosol challenge led to a specific reduction of the pro-inflammatory cytokine IL-5 while other pro-inflammatory or anti-inflammatory cytokines were unaffected [81]. In the same model of inflammation, Ameredes and colleagues [82] showed a CO-induced, cGMP-dependent reduction of airway hyper-responsiveness.

In experimental models of lung ischemia and reperfusion, including transplantation, inhaled CO has anti-inflammatory and anti-apoptotic effects [54,63,83-86]. The p38 MAPK pathway and downstream target genes, such as that for early growth response-1 (Egr-1), seem to play important roles in mediating the CO effects [84].

Mechanical ventilation may cause profound lung injury and inflammatory responses. Dolinay and colleagues [87] described a CO-mediated suppression of tumor necrosis factor (TNF)-alpha release and neutrophil recruitment and postulated an involvement of the p38 MAPK pathway. A study in knock-out mice suggests a key role of Egr-1 as a pro-inflammatory regulator in ventilator-induced lung injury. Moreover, peroxysome proliferator-activated receptor-gamma, an anti-inflammatory nuclear regulator, seems to be involved in the protective effects of CO [88].

In addition to attenuating local lung injury, CO also protects against remote lung injury. After ischemia and reperfusion of the lower extremities, CO significantly reduced ischemia/reperfusion-induced acute lung injury [89]. Pretreatment with inhaled CO reduced pulmonary inflammatory response and provided anti-apoptotic effects in a model of cardiopulmonary bypass in pigs [90].

Liver Effects of CO on the liver have been investigated in models of inflammation- and ischemia/reperfusion-induced hepatocellular injury as well as in burn injury. TNF-alpha-induced hepatocyte cell death in mice was prevented by CO inhalation. CO-induced activation of NF-κB and inducible nitric oxide synthase and nitric oxide-induced HO-1 expression were required for the protective effects [91]. In addition, CO-stimulated liver ATP generation through the activation of sGC was a prerequisite for CO to protect against TNF-alpha-induced apoptosis [92]. In models of liver ischemia and reperfusion, HO-1 induction plays an important role in maintaining hepatocellular integrity [38] and induction of HO-1 before (low flow) ischemia can attenuate the subsequent hepatic injury [32,40]. A role for CO in preventing hypoxia-induced decreases in hepatocyte ATP levels was postulated in a mouse model of hemorrhagic shock and resuscitation [93]. In cold ischemia reperfusion associated with liver transplantation, CO inhalation suppressed the inflammatory response. Downregulation of MEK/ERK1/2 seems to play a role in mediating the protective effects while the NF-κB signaling pathway does not seem to be affected [94]. CO-RM-liberated CO attenuates liver injury in burn mice by mechanisms involving downregulation of pro-inflammatory mediators and suppression of the pro-adhesive phenotype of endothelial cells [95,96].

Intestine The protective effects of CO in the intestine have been investigated in a variety of animal models of postoperative ileus and cold ischemia/reperfusion injury associated with transplantation. The development of postoperative ileus may occur after mild manipulation of the small bowel during surgery, which initiates an inflammatory response within the intestinal muscularis [97] that is characterized by the release of pro-inflammatory mediators, increased expression of adhesion molecules on the vascular endothelium, and recruitment of leukocytes from the systemic circulation [98,99]. Inhalation of CO significantly attenuated the surgically induced molecular inflammatory response and the associated decline in gastrointestinal contractility that is characteristic of postoperative ileus [100,101]. Similar effects could be observed after intraperitoneal injection of CO-saturated Ringer`s lactate solution, possibly in a sGC-dependent manner [102].

Nakao and colleagues [103] provide a large body of evidence that inhaled CO is also protective by improving post-transplant motility and attenuating the inflammatory cytokine response in the syngeneic rat transplant model. In addition, CO is anti-apoptotic and significantly improves animal survival [104]. Similar protective results can be achieved after storage of grafts in University of Wisconsin solution saturated with CO [105].

Vascular diseases Short-term administration of CO has been shown to be protective against vascular injury. CO rescued the pro-thrombotic phenotype of *Hmox1* deficiency during oxidative stress [106]. Intravenous injection of CO-saturated saline produced vasodilatation and improved microvascular hemodynamics in a hamster skinfold window chamber preparation, possibly via increased cardiac output and local cGMP content [107]. Otterbein and colleagues [55] described a beneficial effect of inhaled CO in preventing arteriosclerotic lesions that occur following aorta transplantation.

Heart Experimental models of heart transplantation or cardiopulmonary bypass have been used to investigate CO effects on accompanying organ injury. CO reduced ischemia/reperfusion injury and cardiac rejection of mouse to rat cardiac transplants via anti-apoptotic, anti-inflammatory and vasodilatory mechanisms, and suppression of platelet aggregation and fibrinolysis [65]. Treatment of the donor (CO inhalation) and graft (CO-saturated storage solution) but not the recipient protected against ischemia/reperfusion injury via anti-apoptotic mechanisms [108]. In contrast, low-dose CO inhalation of the recipient after transplantation effectively ameliorated heart allograft rejection via downregulation of pro-inflammatory mediators [109].

In a clinically relevant model of cardiopulmonary bypass surgery in pigs, treatment with CO improved cardiac energetics, prevented edema formation and apoptosis, and facilitated recovery [110]. In a rat model of ischemia/reperfusion injury induced by occlusion of the left anterior descending coronary artery, pre-exposure to CO significantly reduced infarct size and migration of macrophages into infarct areas. In addition, TNF-alpha expression was reduced.

The protective effects were mediated by CO-induced activation of p38 MAPK, protein kinase B (Akt), endothelial nitric oxide synthase, and cGMP in the myocardium [111].

Kidney   Most of the studies of CO effects in kidneys concentrate on models of cold ischemia/reperfusion injury in transplantation. Ischemia/reperfusion injury of kidney grafts is one of the major deleterious factors affecting successful renal transplantation. Renal ischemia/reperfusion injury causes delayed graft function and plays a significant role in the development of chronic allograft nephropathy [112,113]. Exposure to low concentrations of CO prevented fibroinflammatory changes associated with chronic allograft nephropathy and preserved long-term renal allograft function [114]. Storage of kidneys with cold preservation solutions containing CO-RMs also improved their function upon reperfusion [115]. Hypoxia-inducible factor-1-mediated upregulation of vascular endothelial growth factor seems to contribute to the protective mechanisms [116]. Nakao and colleagues [117] provide evidence that prevention of cytochrome P450 degradation, maintenance of normal intracellular heme levels and a reduction of lipid peroxidation participate in the protective effects of CO-RMs during storage of kidney grafts.

Systemic inflammation   As a model of systemic inflammation, lipopolysaccharide (LPS)-induced inflammatory response and organ injury has widely been used to study protective CO-mediated effects. In rodents and pigs injected with LPS, inhalation of CO leading to $14.08 \pm 1.34\%$ COHb significantly reduced LPS-induced cytokine response [118,119] and improved long-term survival [120]. Further mechanisms of CO-mediated protection against LPS-induced multiple injury in rats have been described and include anti-oxidative, anti-inflammatory and anti-apoptotic effects, and up-regulation of HO-1 expression [121]. In contrast, in a randomized, controlled study in pigs, CO exposure did not alter LPS-induced levels of pro- and anti-inflammatory cytokines [122]. The lack of protective effects observed in this study might possibly be explained by the low level of COHb measured (5% compared to 14%) [118].

Clinical studies
While a large body of experimental evidence suggests the potential of low amounts of inhaled CO to protect the lungs and systemic organs and tissues against oxidative and inflammatory insults, only a few studies on therapeutic applications of CO inhalation in humans have been published.

In a randomized, double-blinded, placebo-controlled, two-way cross-over trial experimental endotoxemia was induced in healthy volunteers by injection of 2 ng/kg LPS. The potential anti-inflammatory effects of CO inhalation were investigated by inhalation of 500 ppm CO (leading to an increase in COHb from 1.2% to 7%) versus synthetic air as a placebo for 1 h. CO inhalation had no effect on the inflammatory response as measured by systemic cytokine production (TNF-alpha, IL-6, IL-8, IL-1α and IL-1β) [123]. In this study, no adverse side effects of CO inhalation were observed.

This study is in contrast to the above described results obtained in most experimental models of endotoxemia. Possible explanations for this discrepancy could be that blood from different species has different affinities for CO, different COHb half-lives, different hemoglobin CO saturation points (different COHb levels at the same CO concentration), or different basic physiologies, such as heart rate.

COPD is characterized by an inflammatory and oxidative stress response. Furthermore, COPD is accompanied by increased COHb levels that correlate with exhaled CO [124]. However, the endogenous CO release might not be sufficient to protect against the development and progression of COPD. In a randomized, placebo-controlled, cross-over study 20 ex-smoking patients with stable COPD were examined to assess safety, feasibility, and potential anti-inflammatory effects of CO inhalation. Inhalation of 100 to 125 ppm CO for 2 h per day on 4 consecutive days led to a maximal individual COHb level of 4.5%. In two patients, exacerbations of COPD occurred during or after the CO inhalation period; otherwise the treatment was well tolerated. The primary study endpoint was sputum neutrophil counts. Although there was a trend towards reduction in sputum eosinophils and improvement of bronchial responsiveness, no significant therapeutic effects were observed [125]. The results of this pilot study are interesting, since they provide some evidence for a potential therapeutic use of inhaled CO. However, whether CO inhalation increases the risk of COPD exacerbations needs to be determined.

One clinical study investigating the effects of low amounts of inhaled CO is currently in progress [126]. A single blinded, randomized, placebo controlled phase I study in healthy subjects investigates the potential of inhaled carbon monoxide in preventing lung inflammatory responses following local endotoxin instillation. The study is ongoing, but currently not recruiting participants.

Conclusion
CO has long been regarded solely as a toxic environmental or endogenous waste product. In addition to cytoprotective properties of endogenous CO, recent evidence strongly suggests protective effects of low concentrations of exogenous CO under pathophysiological conditions such as organ transplantation, ischemia/reperfusion, inflammation, sepsis, or shock states. Studies in humans are scarce and so far do not support the promising results observed in pre-clinical experimental studies. A potential beneficial effect of exogenous CO may highly depend on the pathological condition, the mode, time point and duration of application, the administered concentration, and on the target tissue. Further randomized, controlled clinical trials are needed to clarify whether exogenous application of CO, either by inhalation or intravenous

application of CO-RMs, may become a safe and effective preventive and therapeutic tool to treat pathophysiological conditions associated with inflammatory or oxidative stress.

Competing interests
The authors declare that they have no competing interests

Acknowledgements

References
1. Rodkey FL, O'Neal JD, Collison HA, Uddin DE: **Relative affinity of hemoglobin S and hemoglobin A for carbon monoxide and oxygen.** *Clin Chem* 1974, **20:**83-84.
2. Stewart RD: **The effect of carbon monoxide on humans.** *Annu Rev Pharmacol* 1975, **15:**409-423.
3. Piantadosi CA: **Biological chemistry of carbon monoxide.** *Antioxid Redox Signal* 2002, **4:**259-270.
4. Clayton CE, Carraway MS, Suliman HB, Thalmann ED, Thalmann KN, Schmechel DE, Piantadosi CA: **Inhaled carbon monoxide and hyperoxic lung injury in rats.** *Am J Physiol Lung Cell Mol Physiol* 2001, **281:**L949-L957.
5. Hampson NB, Hauff NM: **Carboxyhemoglobin levels in carbon monoxide poisoning: do they correlate with the clinical picture?** *Am J Emerg Med* 2008, **26:**665-669.
6. Foresti R, Bani-Hani MG, Motterlini R: **Use of carbon monoxide as a therapeutic agent: promises and challenges.** *Intensive Care Med* 2008, **34:**649-658.
7. Turino GM: **Effect of carbon monoxide on the cardiorespiratory system. Carbon monoxide toxicity: physiology and biochemistry.** *Circulation* 1981, **63:**253A-259A.
8. Van Meter KW: **Carbon monoxide poisoning.** In *Emergency Medicine*. Edited by Tintinalli JE, Kelen GD, Stapczynski JS. New York: McGraw Hill: New York; 2003:1238-1242.
9. Allred EN, Bleecker ER, Chaitman BR, Dahms TE, Gottlieb SO, Hackney JD, Pagano M, Selvester RH, Walden SM, Warren J: **Short-term effects of carbon monoxide exposure on the exercise performance of subjects with coronary artery disease.** *N Engl J Med* 1989, **321:**1426-1432.
10. **Carbon monoxide-related deaths - United States, 1999-2004.** *MMWR Morb Mortal Wkly Rep* 2007, **56:**1309-1312.
11. Thom SR: **Hyperbaric-oxygen therapy for acute carbon monoxide poisoning.** *N Engl J Med* 2002, **347:**1105-1106.
12. Juurlink DN, Buckley NA, Stanbrook MB, Isbister GK, Bennett M, McGuigan MA: **Hyperbaric oxygen for carbon monoxide poisoning.** *Cochrane Database Syst Rev* 2005, CD002041.
13. **One vs. Three Hyperbaric Oxygen Treatments for Acute Carbon Monoxide Poisoning (1V3CORCT)** [http://www.clinicaltrials.gov/ct2/show/NCT00465855?term=co+poisoning&rank=1]
14. Loennechen JP, Nilsen OG, Arbo I, Aadahl P, Nilsen T, Waldum HL, Sandvik AK, Ellingsen O: **Chronic exposure to carbon monoxide and nicotine: endothelin ET(A) receptor antagonism attenuates carbon monoxide-induced myocardial hypertrophy in rat.** *Toxicol Appl Pharmacol* 2002, **178:**8-14.
15. Coburn RF, Blakemore WS, Forster RE: **Endogenous carbon monoxide production in man.** *J Clin Invest* 1963, **42:**1172-1178.
16. Tenhunen R, Marver HS, Schmid R: **The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase.** *Proc Natl Acad Sci USA* 1968, **61:**748-755.
17. Maines MD, Kappas A: **Cobalt induction of hepatic heme oxygenase; with evidence that cytochrome P-450 is not essential for this enzyme activity.** *Proc Natl Acad Sci USA* 1974, **71:**4293-4297.
18. Yoshida T, Takahashi S, Kikuchi G: **Partial purification and reconstitution of the heme oxygenase system from pig spleen microsomes.** *J Biochem* 1974, **75:**1187-1191.
19. Maines MD, Trakshel GM, Kutty RK: **Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible.** *J Biol Chem* 1986, **261:**411-419.
20. Trakshel GM, Kutty RK, Maines MD: **Purification and characterization of the major constitutive form of testicular heme oxygenase. The noninducible isoform.** *J Biol Chem* 1986, **261:**11131-11137.
21. McCoubrey WK Jr, Huang TJ, Maines MD: **Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3.** *Eur J Biochem* 1997, **247:**725-732.
22. Hayashi S, Omata Y, Sakamoto H, Higashimoto Y, Hara T, Sagara Y, Noguchi M: **Characterization of rat heme oxygenase-3 gene. Implication of processed pseudogenes derived from heme oxygenase-2 gene.** *Gene* 2004, **336:**241-250.
23. Maines MD: **The heme oxygenase system: a regulator of second messenger gases.** *Annu Rev Pharmacol Toxicol* 1997, **37:**517-554.
24. Bauer M, Bauer I: **Heme oxygenase-1: redox regulation and role in the hepatic response to oxidative stress.** *Antioxid Redox Signal* 2002, **4:**749-758.
25. Ryter SW, Alam J, Choi AM: **Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications.** *Physiol Rev* 2006, **86:**583-650.
26. Loboda A, Jazwa A, Grochot-Przeczek A, Rutkowski AJ, Cisowski J, Agarwal A, Jozkowicz A, Dulak J: **Heme oxygenase-1 and the vascular bed: from molecular mechanisms to therapeutic opportunities.** *Antioxid Redox Signal* 2008, **10:**1767-1812.
27. Ferrandiz ML, Devesa I: **Inducers of heme oxygenase-1.** *Curr Pharm Des* 2008, **14:**473-486.
28. Bauer M, Huse K, Settmacher U, Claus RA: **The heme oxygenase-carbon monoxide system: regulation and role in stress response and organ failure.** *Intensive Care Med* 2008, **34:**640-648.
29. Bauer I, Wanner GA, Rensing H, Alte C, Miescher EA, Wolf B, Pannen BH, Clemens MG, Bauer M: **Expression pattern of heme oxygenase isoenzymes 1 and 2 in normal and stress-exposed rat liver.** *Hepatology* 1998, **27:**829-838.
30. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, Koizumi S: **Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency.** *J Clin Invest* 1999, **103:**129-135.
31. Kawashima A, Oda Y, Yachie A, Koizumi S, Nakanishi I: **Heme oxygenase-1 deficiency: the first autopsy case.** *Hum Pathol* 2002, **33:**125-130.
32. Kubulus D, Rensing H, Paxian M, Thierbach JT, Meisel T, Redl H, Bauer M, Bauer I: **Influence of heme-based solutions on stress protein expression and organ failure after hemorrhagic shock.** *Crit Care Med* 2005, **33:**629-637.
33. Kubulus D, Mathes A, Pradarutti S, Raddatz A, Heiser J, Pavlidis D, Wolf B, Bauer I, Rensing H: **Hemin arginate-induced heme oxygenase 1 expression improves liver microcirculation and mediates an anti-inflammatory cytokine response after hemorrhagic shock.** *Shock* 2008, **29:**583-590.
34. Mersmann J, Tran N, Zacharowski PA, Grotemeyer D, Zacharowski K: **Rosiglitazone is cardioprotective in a murine model of myocardial I/R.** *Shock* 2008, **30:**64-68.
35. Nath KA, Balla G, Vercellotti GM, Balla J, Jacob HS, Levitt MD, Rosenberg ME: **Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat.** *J Clin Invest* 1992, **90:**267-270.
36. Otterbein L, Sylvester SL, Choi AM: **Hemoglobin provides protection against lethal endotoxemia in rats: the role of heme oxygenase-1.** *Am J Respir Cell Mol Biol* 1995, **13:**595-601.
37. Pannen BH, Kohler N, Hole B, Bauer M, Clemens MG, Geiger KK: **Protective role of endogenous carbon monoxide in hepatic microcirculatory dysfunction after hemorrhagic shock in rats.** *J Clin Invest* 1998, **102:**1220-1228.

38. Rensing H, Bauer I, Datene V, Patau C, Pannen BH, Bauer M: **Differential expression pattern of heme oxygenase-1/heat shock protein 32 and nitric oxide synthase-II and their impact on liver injury in a rat model of hemorrhage and resuscitation.** *Crit Care Med* 1999, **27:**2766-2775.

39. Rossler OG, Bauer I, Chung HY, Thiel G: **Glutamate-induced cell death of immortalized murine hippocampal neurons: neuroprotective activity of heme oxygenase-1, heat shock protein 70, and sodium selenite.** *Neurosci Lett* 2004, **362:**253-257.

40. Schmidt R, Tritschler E, Hoetzel A, Loop T, Humar M, Halverscheid L, Geiger KK, Pannen BH: **Heme oxygenase-1 induction by the clinically used anesthetic isoflurane protects rat livers from ischemia/reperfusion injury.** *Ann Surg* 2007, **245:**931-942.

41. Tullius SG, Nieminen-Kelhä M, Buelow R, Reutzel-Selke A, Martins PN, Pratschke J, Bachmann U, Lehmann M, Southard D, Iyer S, Schmidbauer G, Sawitzki B, Reinke P, Neuhaus P, Volk HD: **Inhibition of ischemia/reperfusion injury and chronic graft deterioration by a single-donor treatment with cobalt-protoporphyrin for the induction of heme oxygenase-1.** *Transplantation* 2002, **74:**591-598.

42. Applegate LA, Luscher P, Tyrrell RM: **Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells.** *Cancer Res* 1991, **51:**974-978.

43. Balla G, Vercellotti GM, Muller-Eberhard U, Eaton J, Jacob HS: **Exposure of endothelial cells to free heme potentiates damage mediated by granulocytes and toxic oxygen species.** *Lab Invest* 1991, **64:**648-655.

44. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN: **Bilirubin is an antioxidant of possible physiological importance.** *Science* 1987, **235:**1043-1046.

45. Djousse L, Rothman KJ, Cupples LA, Levy D, Ellison RC: **Effect of serum albumin and bilirubin on the risk of myocardial infarction (the Framingham Offspring Study).** *Am J Cardiol* 2003, **91:**485-488.

46. Eisenstein RS, Garcia-Mayol D, Pettingell W, Munro HN: **Regulation of ferritin and heme oxygenase synthesis in rat fibroblasts by different forms of iron.** *Proc Natl Acad Sci USA* 1991, **88:**688-692.

47. Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, Vercellotti GM: **Ferritin: a cytoprotective antioxidant strategem of endothelium.** *J Biol Chem* 1992, **267:**18148-18153.

48. Furchgott RF, Jothianandan D: **Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light.** *Blood Vessels* 1991, **28:**52-61.

49. Morita T, Mitsialis SA, Koike H, Liu Y, Kourembanas S: **Carbon monoxide controls the proliferation of hypoxic vascular smooth muscle cells.** *J Biol Chem* 1997, **272:**32804-32809.

50. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH: **Carbon monoxide: a putative neural messenger.** *Science* 1993, **259:**381-384.

51. Cardell LO, Ueki IF, Stjärne P, Agusti C, Takeyama K, Lindén A, Nadel JA: **Bronchodilatation in vivo by carbon monoxide, a cyclic GMP related messenger.** *Br J Pharmacol* 1998, **124:**1065-1068.

52. Suematsu M, Kashiwagi S, Sano T, Goda N, Shinoda Y, Ishimura Y: **Carbon monoxide as an endogenous modulator of hepatic vascular perfusion.** *Biochem Biophys Res Commun* 1994, **205:**1333-1337.

53. Brune B, Ullrich V: **Inhibition of platelet aggregation by carbon monoxide is mediated by activation of guanylate cyclase.** *Mol Pharmacol* 1987, **32:**497-504.

54. Fujita T, Toda K, Karimova A, Yan SF, Naka Y, Yet SF, Pinsky DJ: **Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis.** *Nat Med* 2001, **7:**598-604.

55. Otterbein LE, Zuckerbraun BS, Haga M, Liu F, Song R, Usheva A, Stachulak C, Bodyak N, Smith RN, Csizmadia E, Tyagi S, Akamatsu Y, Flavell RJ, Billiar TR, Tzeng E, Bach FH, Choi AM, Soares MP: **Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury.** *Nat Med* 2003, **9:**183-190.

56. Utz J, Ullrich V: **Carbon monoxide relaxes ileal smooth muscle through activation of guanylate cyclase.** *Biochem Pharmacol* 1991, **41:**1195-1201.

57. Wang R, Wu L, Wang Z: **The direct effect of carbon monoxide on KCa channels in vascular smooth muscle cells.** *Pflugers Arch* 1997, **434:**285-291.

58. Ryter SW, Otterbein LE, Morse D, Choi AM: **Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance.** *Mol Cell Biochem* 2002, **234-235:**249-263.

59. Kim HP, Ryter SW, Choi AM: **CO as a cellular signaling molecule.** *Annu Rev Pharmacol Toxicol* 2006, **46:**411-449.

60. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, Choi AM: **Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway.** *Nat Med* 2000, **6:**422-428.

61. Kim HP, Wang X, Zhang J, Suh GY, Benjamin IJ, Ryter SW, Choi AM: **Heat shock protein-70 mediates the cytoprotective effect of carbon monoxide: involvement of p38 beta MAPK and heat shock factor-1.** *J Immunol* 2005, **175:**2622-2629.

62. Kim HP, Wang X, Nakao A, Kim SI, Murase N, Choi ME, Ryter SW, Choi AM: **Caveolin-1 expression by means of p38beta mitogen-activated protein kinase mediates the antiproliferative effect of carbon monoxide.** *Proc Natl Acad Sci USA* 2005, **102:**11319-11324.

63. Zhang X, Shan P, Alam J, Fu XY, Lee PJ: **Carbon monoxide differentially modulates STAT1 and STAT3 and inhibits apoptosis via a phosphatidylinositol 3-kinase/Akt and p38 kinase-dependent STAT3 pathway during anoxia-reoxygenation injury.** *J Biol Chem* 2005, **280:**8714-8721.

64. Kim HS, Loughran PA, Rao J, Billiar TR, Zuckerbraun BS: **Carbon monoxide activates NF-kappaB via ROS generation and Akt pathways to protect against cell death of hepatocytes.** *Am J Physiol Gastrointest Liver Physiol* 2008, **295:**G146-G152.

65. Sato K, Balla J, Otterbein L, Smith RN, Brouard S, Lin Y, Csizmadia E, Sevigny J, Robson SC, Vercellotti G, Choi AM, Bach FH, Soares MP: **Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants.** *J Immunol* 2001, **166:**4185-4194.

66. Abraham NG, Asija A, Drummond G, Peterson S: **Heme oxygenase-1 gene therapy: recent advances and therapeutic applications.** *Curr Gene Ther* 2007, **7:**89-108.

67. Stupfel M, Bouley G: **Physiological and biochemical effects on rats and mice exposed to small concentrations of carbon monoxide for long periods.** *Ann N Y Acad Sci* 1970, **174:**342-368.

68. Herrmann WA: **100 years of metal carbonyls: a serendipitous chemical discovery of major scientific and industrial impact.** *J Organomet Chem* 1990, **383:**21-44.

69. Motterlini R: **Carbon monoxide-releasing molecules (CO-RMs): vasodilatory, anti-ischaemic and anti-inflammatory activities.** *Biochem Soc Trans* 2007, **35:**1142-1146.

70. Motterlini R, Mann BE, Foresti R: **Therapeutic applications of carbon monoxide-releasing molecules.** *Expert Opin Investig Drugs* 2005, **14:**1305-1318.

71. Stewart RD, Peterson JE, Baretta ED, Bachand RT, Hosko MJ, Herrmann AA: **Experimental human exposure to carbon monoxide.** *Arch Environ Health* 1970, **21:**154-164.

72. Wu L, Wang R: **Carbon monoxide: endogenous production, physiological functions, and pharmacological applications.** *Pharmacol Rev* 2005, **57:**585-630.

73. Schober P, Koch A, Zacharowski K, Loer SA: **[Carbon monoxide: toxic molecule with antiinflammatory and cytoprotective properties].** *Anasthesiol Intensivmed Notfallmed Schmerzther* 2006, **41:**140-149.

74. Hoetzel A, Schmidt R: **[Carbon monoxide - poison or potential therapeutic?].** *Anaesthesist* 2006, **55:**1068-1079.

75. Hoetzel A, Dolinay T, Schmidt R, Choi AM, Ryter SW: **Carbon monoxide in sepsis.** *Antioxid Redox Signal* 2007, **9:**2013-2026.

76. Otterbein LE, Mantell LL, Choi AM: **Carbon monoxide provides protection against hyperoxic lung injury.** *Am J Physiol* 1999, **276:**L688-L694.

77. Otterbein LE, Otterbein SL, Ifedigbo E, Liu F, Morse DE, Fearns C, Ulevitch RJ, Knickelbein R, Flavell RA, Choi AM: **MKK3 mitogen-activated protein kinase pathway mediates carbon monoxide-induced protection against oxidant-induced lung injury.** *Am J Pathol* 2003, **163:**2555-2563.

78. Dubuis E, Potier M, Wang R, Vandier C: **Continuous inhalation of carbon monoxide attenuates hypoxic pulmonary hypertension development presumably through activation of BKCa channels.** *Cardiovasc Res* 2005, **65:**751-761.

79. Zuckerbraun BS, Chin BY, Wegiel B, Billiar TR, Czsimadia E, Rao J, Shimoda L, Ifedigbo E, Kanno S, Otterbein LE: **Carbon monoxide reverses established pulmonary hypertension.** *J Exp Med* 2006, **203:**2109-2119.

80. Nemzek JA, Fry C, Abatan O: **Low-dose carbon monoxide treatment attenuates early pulmonary neutrophil recruitment after acid aspiration.** *Am J Physiol Lung Cell Mol Physiol* 2008, **294:** L644-L653.

81. Chapman JT, Otterbein LE, Elias JA, Choi AM: **Carbon monoxide attenuates aeroallergen-induced inflammation in mice.** *Am J Physiol Lung Cell Mol Physiol* 2001, **281:**L209-L216.

82. Ameredes BT, Otterbein LE, Kohut LK, Gligonic AL, Calhoun WJ, Choi AM: **Low-dose carbon monoxide reduces airway hyper-responsiveness in mice.** *Am J Physiol Lung Cell Mol Physiol* 2003, **285:**L1270-L1276.

83. Kohmoto J, Nakao A, Stolz DB, Kaizu T, Tsung A, Ikeda A, Shimizu H, Takahashi T, Tomiyama K, Sugimoto R, Choi AM, Billiar TR, Murase N, McCurry KR: **Carbon monoxide protects rat lung transplants from ischemia-reperfusion injury via a mechanism involving p38 MAPK pathway.** *Am J Transplant* 2007, **7:**2279-2290.

84. Mishra S, Fujita T, Lama VN, Nam D, Liao H, Okada M, Minamoto K, Yoshikawa Y, Harada H, Pinsky DJ: **Carbon monoxide rescues ischemic lungs by interrupting MAPK-driven expression of early growth response 1 gene and its downstream target genes.** *Proc Natl Acad Sci USA* 2006, **103:**5191-5196.

85. Song R, Kubo M, Morse D, Zhou Z, Zhang X, Dauber JH, Fabisiak J, Alber SM, Watkins SC, Zuckerbraun BS, Otterbein LE, Ning W, Oury TD, Lee PJ, McCurry KR, Choi AM: **Carbon monoxide induces cytoprotection in rat orthotopic lung transplantation via anti-inflammatory and anti-apoptotic effects.** *Am J Pathol* 2003, **163:**231-242.

86. Zhang X, Shan P, Alam J, Davis RJ, Flavell RA, Lee PJ: **Carbon monoxide modulates Fas/Fas ligand, caspases, and Bcl-2 family proteins via the p38alpha mitogen-activated protein kinase pathway during ischemia-reperfusion lung injury.** *J Biol Chem* 2003, **278:**22061-22070.

87. Dolinay T, Szilasi M, Liu M, Choi AM: **Inhaled carbon monoxide confers antiinflammatory effects against ventilator-induced lung injury.** *Am J Respir Crit Care Med* 2004, **170:**613-620.

88. Hoetzel A, Dolinay T, Vallbracht S, Zhang Y, Kim HP, Ifedigbo E, Alber S, Kaynar AM, Schmidt R, Ryter SW, Choi AM: **Carbon monoxide protects against ventilator-induced lung injury via PPAR-gamma and inhibition of Egr-1.** *Am J Respir Crit Care Med* 2008, **177:**1223-1232.

89. Boutros C, Zegdi R, Lila N, Cambillau M, Fornes P, Carpentier A, Fabini JN: **Carbon monoxide can prevent acute lung injury observed after ischemia reperfusion of the lower extremities.** *J Surg Res* 2007, **143:**437-442.

90. Goebel U, Siepe M, Mecklenburg A, Stein P, Roesslein M, Schwer CI, Schmidt R, Doenst T, Geiger KK, Pahl HL, Schlensak C, Loop T: **Carbon monoxide inhalation reduces pulmonary inflammatory response during cardiopulmonary bypass in pigs.** *Anesthesiology* 2008, **108:**1025-1036.

91. Zuckerbraun BS, Billiar TR, Otterbein SL, Kim PK, Liu F, Choi AM, Bach FH, Otterbein LE: **Carbon monoxide protects against liver failure through nitric oxide-induced heme oxygenase 1.** *J Exp Med* 2003, **198:**1707-1716.

92. Tsui TY, Obed A, Siu YT, Yet SF, Prantl L, Schlitt HJ, Fan ST: **Carbon monoxide inhalation rescues mice from fulminant hepatitis through improving hepatic energy metabolism.** *Shock* 2007, **27:**165-171.

93. Zuckerbraun BS, McCloskey CA, Gallo D, Liu F, Ifedigbo E, Otterbein LE, Billiar TR: **Carbon monoxide prevents multiple organ injury in a model of hemorrhagic shock and resuscitation.** *Shock* 2005, **23:**527-532.

94. Kaizu T, Ikeda A, Nakao A, Tsung A, Toyokawa H, Ueki S, Geller DA, Murase N: **Protection of transplant-induced hepatic ischemia/reperfusion injury with carbon monoxide via MEK/ERK1/2 pathway downregulation.** *Am J Physiol Gastrointest Liver Physiol* 2008, **294:**G236-G244.

95. Sun BW, Chen ZY, Chen X, Liu C: **Attenuation of leukocytes sequestration by carbon monoxide-releasing molecules: liberated carbon monoxide in the liver of thermally injured mice.** *J Burn Care Res* 2007, **28:**173-181.

96. Sun BW, Sun Y, Sun ZW, Chen X: **CO liberated from CORM-2 modulates the inflammatory response in the liver of thermally injured mice.** *World J Gastroenterol* 2008, **14:**547-553.

97. Kalff JC, Carlos TM, Schraut WH, Billiar TR, Simmons RL, Bauer AJ: **Surgically induced leukocytic infiltrates within the rat intestinal muscularis mediate postoperative ileus.** *Gastroenterology* 1999, **117:**378-387.

98. Kalff JC, Hierholzer C, Tsukada K, Billiar TR, Bauer AJ: **Hemorrhagic shock results in intestinal muscularis intercellular adhesion molecule (ICAM-1) expression, neutrophil infiltration, and smooth muscle dysfunction.** *Arch Orthop Trauma Surg* 1999, **119:**89-93.

99. Turler A, Schwarz NT, Turler E, Kalff JC, Bauer AJ: **MCP-1 causes leukocyte recruitment and subsequently endotoxemic ileus in rat.** *Am J Physiol Gastrointest Liver Physiol* 2002, **282:**G145-G155.

100. Moore BA, Otterbein LE, Turler A, Choi AM, Bauer AJ: **Inhaled carbon monoxide suppresses the development of postoperative ileus in the murine small intestine.** *Gastroenterology* 2003, **124:**377-391.

101. Moore BA, Overhaus M, Whitcomb J, Ifedigbo E, Choi AM, Otterbein LE, Bauer AJ: **Brief inhalation of low-dose carbon monoxide protects rodents and swine from postoperative ileus.** *Crit Care Med* 2005, **33:**1317-1326.

102. Nakao A, Schmidt J, Harada T, Tsung A, Stoffels B, Cruz RJ Jr, Kohmoto J, Peng X, Tomiyama K, Murase N, Bauer AJ, Fink MP: **A single intraperitoneal dose of carbon monoxide-saturated ringer's lactate solution ameliorates postoperative ileus in mice.** *J Pharmacol Exp Ther* 2006, **319:**1265-1275.

103. Nakao A, Moore BA, Murase N, Liu F, Zuckerbraun BS, Bach FH, Choi AM, Nalesnik MA, Otterbein LE, Bauer AJ: **Immunomodulatory effects of inhaled carbon monoxide on rat syngeneic small bowel graft motility.** *Gut* 2003, **52:**1278-1285.

104. Nakao A, Kimizuka K, Stolz DB, Neto JS, Kaizu T, Choi AM, Uchiyama T, Zuckerbraun BS, Nalesnik MA, Otterbein LE, Murase N: **Carbon monoxide inhalation protects rat intestinal grafts from ischemia/reperfusion injury.** *Am J Pathol* 2003, **163:** 1587-1598.

105. Nakao A, Toyokawa H, Tsung A, Nalesnik MA, Stolz DB, Kohmoto J, Ikeda A, Tomiyama K, Harada T, Takahashi T, Yang R, Fink MP, Morita K, Choi AM, Murase N: **Ex vivo application of carbon monoxide in University of Wisconsin solution to prevent intestinal cold ischemia/reperfusion injury.** *Am J Transplant* 2006, **6:**2243-2255.

106. True AL, Olive M, Boehm M, San H, Westrick RJ, Raghavachari N, Xu X, Lynn EG, Sack MN, Munson PJ, Gladwin MT, Nabel EG: **Heme oxygenase-1 deficiency accelerates formation of arterial thrombosis through oxidative damage to the endothelium, which is rescued by inhaled carbon monoxide.** *Circ Res* 2007, **101:**893-901.

107. Hangai-Hoger N, Tsai AG, Cabrales P, Suematsu M, Intaglietta M: **Microvascular and systemic effects following top load administration of saturated carbon monoxide-saline solution.** *Crit Care Med* 2007, **35:**1123-1132.

108. Akamatsu Y, Haga M, Tyagi S, Yamashita K, Graça-Souza AV, Ollinger R, Czsimadia E, May GA, Ifedigbo E, Otterbein LE, Bach FH, Soares MP: **Heme oxygenase-1-derived carbon monoxide protects hearts from transplant associated ischemia reperfusion injury.** *FASEB J* 2004, **18:**771-772.

109. Nakao A, Toyokawa H, Abe M, Kiyomoto T, Nakahira K, Choi AM, Nalesnik MA, Thomson AW, Murase N: **Heart allograft protection with low-dose carbon monoxide inhalation: effects on inflammatory mediators and alloreactive T-cell responses.** *Transplantation* 2006, **81:**220-230.

110. Lavitrano M, Smolenski RT, Musumeci A, Maccherini M, Slominska E, Di Florio E, Bracco A, Mancini A, Stassi G, Patti M, Giovannoni R, Froio A, Simeone F, Forni M, Bacci ML, D'Alise G, Cozzi E, Otterbein LE, Yacoub MH, Bach FH, Calise F: **Carbon monoxide improves cardiac energetics and safeguards the heart during reperfusion after cardiopulmonary bypass in pigs.** *FASEB J* 2004, **18:**1093-1095.

111. Fujimoto H, Ohno M, Ayabe S, Kobayashi H, Ishizaka N, Kimura H, Yoshida K, Nagai R: **Carbon monoxide protects against cardiac ischemia-reperfusion injury in vivo via MAPK and Akt-eNOS pathways.** *Arterioscler Thromb Vasc Biol* 2004, **24:**1848-1853.

112. Tullius SG, Heemann U, Hancock WW, Azuma H, Tilney NL: **Long-term kidney isografts develop functional and morphologic changes that mimic those of chronic allograft rejection.** *Ann Surg* 1994, **220:**425-432.

113. Gueler F, Gwinner W, Schwarz A, Haller H: **Long-term effects of acute ischemia and reperfusion injury.** *Kidney Int* 2004, **66:** 523-527.

114. Neto JS, Nakao A, Toyokawa H, Nalesnik MA, Romanosky AJ, Kimizuka K, Kaizu T, Hashimoto N, Azhipa O, Stolz DB, Choi AM, Murase N: **Low-dose carbon monoxide inhalation prevents development of chronic allograft nephropathy.** *Am J Physiol Renal Physiol* 2006, **290:**F324-F334.

115. Sandouka A, Fuller BJ, Mann BE, Green CJ, Foresti R, Motterlini R: **Treatment with CO-RMs during cold storage improves renal function at reperfusion.** *Kidney Int* 2006, **69:**239-247.

116. Faleo G, Neto JS, Kohmoto J, Tomiyama K, Shimizu H, Takahashi T, Wang Y, Sugimoto R, Choi AM, Stolz DB, Carrieri G, McCurry KR, Murase N, Nakao A: **Carbon monoxide ameliorates renal cold ischemia-reperfusion injury with an upregulation of vascular endothelial growth factor by activation of hypoxia-inducible factor.** *Transplantation* 2008, **85:**1833-1840.

117. Nakao A, Faleo G, Shimizu H, Nakahira K, Kohmoto J, Sugimoto R, Choi AM, McCurry KR, Takahashi T, Murase N: **Ex vivo carbon monoxide prevents cytochrome P450 degradation and ischemia/reperfusion injury of kidney grafts.** *Kidney Int* 2008, **74:**1009-1016.

118. Mazzola S, Forni M, Albertini M, Bacci ML, Zannoni A, Gentilini F, Lavitrano M, Bach FH, Otterbein LE, Clement MG: **Carbon monoxide pretreatment prevents respiratory derangement and ameliorates hyperacute endotoxic shock in pigs.** *FASEB J* 2005, **19:**2045-2047.

119. Bilban M, Bach FH, Otterbein SL, Ifedigbo E, d'Avila JC, Esterbauer H, Chin BY, Usheva A, Robson SC, Wagner O, Otterbein LE: **Carbon monoxide orchestrates a protective response through PPARgamma.** *Immunity* 2006, **24:**601-610.

120. Sarady JK, Zuckerbraun BS, Bilban M, Wagner O, Usheva A, Liu F, Ifedigbo E, Zamora R, Choi AM, Otterbein LE: **Carbon monoxide protection against endotoxic shock involves reciprocal effects on iNOS in the lung and liver.** *FASEB J* 2004, **18:**854-856.

121. Liu SH, Xu XR, Ma K, Xu B: **Protection of carbon monoxide inhalation on lipopolysaccharide-induced multiple organ injury in rats.** *Chin Med Sci J* 2007, **22:**169-176.

122. Aberg AM, Abrahamsson P, Johansson G, Haney M, Winso O, Larsson JE: **Does carbon monoxide treatment alter cytokine levels after endotoxin infusion in pigs? A randomized controlled study.** *J Inflamm* 2008, **5:**13.

123. Mayr FB, Spiel A, Leitner J, Marsik C, Germann P, Ullrich R, Wagner O, Jilma B: **Effects of carbon monoxide inhalation during experimental endotoxemia in humans.** *Am J Respir Crit Care Med* 2005, **171:**354-360.

124. Yasuda H, Yamaya M, Nakayama K, Ebihara S, Sasaki T, Okinaga S, Inoue D, Asada M, Nemoto M, Sasaki H: **Increased arterial carboxyhemoglobin concentrations in chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med* 2005, **171:**1246-1251.

125. Bathoorn E, Slebos DJ, Postma DS, Koeter GH, van Oosterhout AJ, van der Toorn M, Boezen HM, Kerstjens HA: **Anti-inflammatory effects of inhaled carbon monoxide in patients with COPD: a pilot study.** *Eur Respir J* 2007, **30:**1131-1137.

126. **Carbon Monoxide to Prevent Lung Inflammation** [http://www.clinicaltrials.gov/ct2/show/NCT00094406?term=inhaled+carbon+monoxide+lung+inflammatory+responses+endotoxin&rank=1]