doi:10.4149/gpb_2012_008

Neither inhalative nor intravenous application of carbon monoxide modifies gastric mucosal oxygenation

Christian Vollmer, Ingo Schwartges, Katja Obermiller, Christopher Beck and Olaf Picker

Department of Anesthesiology, University Hospital Duesseldorf, Duesseldorf, Germany

Abstract. This study was designed to compare the effects of different ways of administering carbon monoxide (intravenous and inhalative) on gastric mucosal oxygenation in a canine model of hemorrhage. Six chronically instrumented dogs were repeatedly anesthetized and randomized to each of the following protocols: In a first series the animals were ventilated either with 100 ppm carbon monoxide (CO) or without followed by hemorrhage and re-transfusion. In a second series a saturated CO solution was infused, compared to normal saline, again followed by hemorrhage and re-transfusion. In a control series, animals received either CO-saline or saline without any further intervention. Microvascular oxygenation of the gastric mucosa (μHbO_2) was assessed continuously by tissue reflectance spectrophotometry. Cardiac output was measured intermittently and oxygen delivery (DO_2) was calculated. The application of CO, inhalative and intravenous, increased carboxyhemoglobin levels without effect on μHbO_2. Hemorrhage reduced μHbO_2 in all groups, paralleled by a reduction in DO_2 without any differences between groups related to the application of CO. Neither intravenous nor inhalative application of CO alters μHbO_2 during physiological conditions or during hemorrhage. Thus, independent of the application way, low dose CO does not seem to modulate regional mucosal oxygenation in cytoprotective concentrations.

Key words: Carbon monoxide — Gastrointestinal oxygenation — Hemorrhage

Introduction

The gastrointestinal tract is a complex organ system with multiple functions. Apart from being responsible for nutrient absorption it is an important metabolic and immunological system, functioning as an effective barrier against endotoxin and bacteria from the intestinal lumen. To maintain this barrier adequate perfusion and oxygenation of the mucosa are vital (Russellet al. 1995; Trzeciak et al. 2007, 2008).

However, during severe illness (e.g. sepsis or hypovolemia), the blood flow is redistributed to preserve perfusion of more vital organs (i.e. heart and brain) and thus splanchnic oxygenation is impaired early (Jakob and Takala 2000). Impairment of the splanchnic blood flow and microcirculation often persists despite adequate fluid resuscitation sufficient to restore global circulatory variables (Edouard et al. 1994). An impaired mucosal barrier function resulting from insufficient microcirculatory oxygen supply has been shown to enable translocation of bacteria and bacterial toxins into portal venous and local lymphatic circulation (Deitch et al. 2004) and to mediate an inflammatory response syndrome (Chen et al. 2003). Therefore, adequate splanchnic perfusion and in particular oxygenation of the gastrointestinal mucosa are considered crucial for the prevention and therapy of critical illness (Trzeciak et al. 2007, 2008). Thus, growing effort is made to develop strategies to improve splanchnic mucosal oxygenation.

A lot of signalling molecules are known to mediate vasodilation (e.g., NO) and might influence microcirculation and oxygen supply of the splanchnic region. The signalling function of carbon monoxide (CO) just recently emerged, but its impact on gastric mucosal perfusion and oxygenation is promising but yet unknown.

For a long time, CO was only associated with its acute and chronic toxicity on animals and humans. However, CO is produced endogenously by the activity of hemoxygenases (HOs), mainly during hemoglobin degradation. Only a small fraction is derived from the degradation of other heme proteins like myoglobin, catalyse, peroxidases and

Correspondence to: Christian Vollmer, Department of Anesthesiology, University Hospital Duesseldorf, Moorenstrasse 5, D-40225 Duesseldorf, Germany
E-mail: christian.vollmer@uni-duesseldorf.de

cytochromes (Berk et al. 1974[1]). Under pathophysiological conditions additional sources are lipid peroxidation (Vreman et al. 1998) and metabolic activity of intestinal bacteria (Engel et al. 1972). 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 (Bauer and Pannen 2009). Furthermore it acts as transcriptional activator (Aono et al. 2000). Effects like these, mediated by gaseous molecules like CO, do not depend on one specific receptor and accordingly they can produce myriad effects virtually simultaneously (Kajimura et al. 2010). For example, vasoactive effects of CO are mediated *via* activation of sGC/cGMP (Boehning and Snyder 2003), although CO serves as partial antagonist in the presence of NO *in vitro* (Kajimura et al. 2003), and directly *via* the activation of calcium-dependent potassium channels in vascular smooth muscle cells (Wang et al. 1997). This leads to vasodilation and exerts beneficial effects on several organ systems, i.e., the heart (Nishikawa et al. 2004), kidneys (Hosgood et al. 2008), lung (Hartsfield et al. 2004), liver (Pannen et al. 1998), and brain (Kanu et al. 2006).

Those vasodilatory effects are known to affect microcirculation in a hamster window chamber model as well and can also be mediated by low doses of exogenous CO that do not even increase CO-Hb (Hangai-Hoger 2007), which could thus allow a therapeutic use of CO. Currently several means of application are available for exogenous CO (inhalation (Kanten et al. 1983) or intraperitoneal (Gutierrez et al. 1985) and intravenous (Hangai-Hoger 2007) infusion). However, effects might not only depend on systemic concentration but rather depend on the amount of gas actually delivered to the given target, e.g. microvessels of the gastric mucosa. This depends not only on properties of the gas itself, but also on the local environment, e.g. viscosity, temperature or tissue composition and chemical reactions that might consume the gas (Kajimura et al. 2010). Thus, it is yet unknown whether vasodilatory effects of the hamster window chamber model can be transferred to other tissues and hence whether exogenous CO positively affects gastric mucosal oxygenation and if the way of application modifies this effect.

CO attenuated the surgically-induced molecular inflammatory response and prevented a postoperative ileus (Moore et al. 2005). Furthermore, it reduced mortality after intestinal transplantation (Nakao et al. 2003) and during microbial sepsis in mice (Chung et al. 2008) which suggests a beneficial effect of CO in the intestine. This is confirmed by findings that HO-2 is localized in neurons of the myenteric plexus of the gut and colocalized with NO-synthase (Boehning and Snyder 2003) which produces another vasoactive transmitter (NO) in gastrointestinal vessels (Zakhary et al. 1996). More and more studies suggest parallelism between the biological actions and functions of the CO- and NO-generating systems; and their regulation is intimately linked (Maines 1997). Some *in vitro* studies suggest inhibition of NO-synthase by CO, however, this requires high concentrations which cannot be found *in vivo*, unless they could be achieved locally (Kajimura et al. 2010). Still, the HO-CO system might downregulate NO-synthase activity by reducing substrate availability as both HO and NO-synthase use the same substrate, i.e. NADPH. Therefore CO is discussed to be in part a vasoconstrictor as well and different effects at different organs are attributed to different receptor systems and different colocalization with NO-synthase.

During stress and pathological conditions, e.g. hemorrhage, induction of hemeoxygenase is triggered due to ischemia/hypoxia (HO-1) and adrenal glucocorticoids (HO-2) (Maines 1997). In addition, the exogenous application of CO-bound hemoglobin seemed to be beneficial in a model of hemorrhagic shock (Sakai et al. 2009).

These data suggest a modulatory role of CO on microvascular gastric mucosal perfusion and oxygenation. CO might be able to restore and maintain mucosal barrier function in severe illness especially during hemorrhagic shock. Therefore, the aim of this study was to evaluate the impact of CO on gastric mucosal oxygenation under physiological and hemorrhagic conditions and to compare the effectiveness of different ways of application, i.e., intravenous to inhalative.

Materials and Methods

Animals

The data were derived from repetitive experiments on six dogs (female foxhounds, weighing 28 ± 1 kg). Prior to the experiments, food was withheld for 12 h with water ad libitum to ensure complete gastric depletion and to avoid perfusion/oxygenation changes due to digestive activity. Each dog underwent two experimental protocols in a first series and four experimental protocols in a second series in randomized order. Experiments were performed at least 2 weeks apart to prevent carryover effects. The experiments were performed under general anaesthesia (induction of anaesthesia with 4 $mg \cdot kg^{-1}$ propofol, maintenance with sevoflurane, end-tidal concentration 3.0%, 1.5 minimum alveolar concentration (MAC) in dogs (Kazama and Ikeda 1988)). The animals were mechanically ventilated after endotracheal intubation (inspiratory oxygen concentration $F_iO_2 = 0.25$, tidal volume VT = 12.5 $ml \cdot kg^{-1}$) with the respiratory frequency adjusted to achieve normocapnia (end-expiratory concentration of carbon dioxide $etCO_2$ = 35 mmHg), verified by continuous capnography (Capnomac Ultima, Datex Instrumentarium, Helsinki, Finland). During

[1] Citation derived from (Kajimura et al. 2010)

the experiments, the dogs were placed on their right side covered with warming blankets to maintain body temperature within the physiological range for dogs (37–38.5°C, continuous arterial measurement). Throughout the experiments, no extra fluids apart from the interventions were administered to avoid volume effects that could influence tissue perfusion and oxygenation. However, after each blood sample, normal saline was infused three times the sampling volume to maintain the blood volume.

Ethical approval for this study was provided by the local District Governmental Animal Investigation Committee. Animals were treated in accordance with NIH guidelines for animal care.

Measurements

Gastric mucosal oxygenation

Microvascular oxygen saturation (μHbO_2) of the gastric mucosa was continuously assessed by a method of the so-called O2C ("oxygen to see") which assesses μHbO_2 by tissue reflectance spectrophotometry (device name "O2C", LEA Medizintechnik, Gießen, Germany), as detailed previously (Frank et al. 1989; Krug 2006).

Briefly, the flexible light-guide probe is introduced into the stomach non-traumatically *via* an orogastric silicone tube. White light (450–1000 nm) is transmitted to the tissue of interest, in this case the gastric mucosa, *via* the micro-lightguide and the reflected light is then analyzed *in vivo*. Thus, no tissues have to be removed from the dogs and gastric mucosa remains intact. Therefore it is possible to analyse different interventions at the same healthy, undamaged tissue. The wavelength-dependent absorption of the applied white light can be used to calculate the percentage of oxygenated hemoglobin (Kuchenreuther et al. 1996). Common pulse oximeters analyze only two wavelengths (660 and 940 nm) where CO-Hb and oxygenated Hb cannot be separated (Barker and Tremper 1987; Zijlstra et al. 1991). In contrast, the O2C analyses almost the entire spectrum from 500–800 nm (Krug 2006), where absorption spectra of oxyhemoglobin and carboxyhemoglobin differ substantially (Zijlstra and Buursma 1987). Therefore measurement of oxygenated hemoglobin is not affected by low concentrations of CO-Hb.

Since light is totally absorbed in vessels with a diameter >100 µm (Gandjbakhche et al. 1999), only microvascular oxygenation of nutritive vessels of the mucosa is measured. The biggest fraction of the blood volume is stored in venous vessels, therefore mainly postcapillary oxygenation is measured which represents the critical partial pressure of oxygen (pO_2) for ischemia (Siegemund et al. 1999).

The flexible light-guide probe is positioned facing the greater curvature (Scheeren et al. 2002), a site demonstrated to represent the microvascular oxygenation of other gastric and upper intestinal mucosa regions (Temmesfeld-Wollbruck et al. 1998). Online evaluation of the signal quality throughout the experiments allows verification of the correct position of the probe tip. The μHbO_2 values reported are the means of the last 5 min (150 spectra, 2 s each) of the respective intervention under steady state conditions. This method allows detection of splanchnic ischemia with similar precision as laser flowmetry (Leung et al. 1987), intra-vital microscopy (Bellamy et al. 1997) or hydrogen gas clearance (Machens et al. 1995). The non-traumatic instrumentation and in particular non-traumatic access to the gastric mucosa allows the determination of mucosal oxygenation in the absence of surgical stress. This is particularly desirable with respect to the marked alterations surgical stress exerts on splanchnic circulation (Gelman 1976). In this situation reflectance spectrophotometry reliably detects even clinically asymptomatic reductions in mucosal oxygenation (Fournell et al. 2003) and highly correlates with the morphologic severity and extent of gastric mucosal tissue injury (Sato et al. 1986).

Systemic hemodynamics and oxygenation

The aorta was catheterized *via* the left carotid artery for continuous measurement of mean arterial pressure (MAP, Gould-Statham pressure transducers P23ID, Elk Grove, IL, USA) and intermittent withdrawal of blood samples for measurement of blood gas tensions including carboxyhemoglobin (CO-Hb) and acid-base related variables (Rapidlab 860, Bayer AG, Germany). Arterial oxygen content (CaO_2) and systemic oxygen delivery ($DO_2 = CaO_2 \times$ cardiac output) were calculated. Cardiac output was determined *via* transpulmonary thermodilution (PiCCO 4.2 non US, PULSION Medical Systems, Munich, Germany) at the end of each intervention, at least every 30 minutes, as described elsewhere (von Spiegel 1996).

Heart rate (HR) was continuously measured by electrocardiography (Powerlab, ADInstruments, Castle Hill, Australia). All hemodynamic and respiratory variables were recorded on a personal computer after analoge to digital conversion (Powerlab, ADInstruments, Castle Hill, Australia) for later analysis.

Carbon monoxide

Carbon monoxide was either inhaled or infused intravenously. For inhaled application, CO (Kohlenmonoxid 4.7, Linde AG Gas und Engineering, Leuna, Germany) was mixed with oxygen-enriched air ($F_iO_2 = 0.25$) and sevoflurane to achieve an inspiratory concentration of 100 ppm which was continuously measured (Draeger Pac

5000, Draegerwerk AG & Co. KGaA, Luebeck, Germany). To infuse CO normal saline (0.9% NaCl) was bubbled with 100% CO (Linde AG) for 20 minutes at room temperature and atmospheric pressure resulting in a concentration of about 0.93×10^{-4} M (CO = 28.01 molecular weight and 0.0026 g/100 ml solubility in water at 20°C) (Hangai-Hoger 2007). Blood volume (BV) was estimated at 80 ml/kg body weight. The animals received 10 %BV of saline or CO-saline as a single infusion over 10 min.

Hypovolemia

Hypovolemia was induced by hemorrhage (withdrawal of 16 ml kg^{-1} of whole blood over five minutes that is 20% of the estimated total blood volume, *via* a large bore intravenous catheter in a femoral vein and the arterial catheter). Heparinized shed blood was stored and later retransfused using an infusion set with a 200 µm filter.

Experimental program

Following the instrumentation, 30 min were allowed to establish steady state conditions and baseline values were recorded before the animals were randomized to the respective protocol (Fig. 1).

Inhaled CO

After baseline recordings, the animals were ventilated either with 100 ppm CO ($inhal_{100ppm/hem}$) or without ($inhal_{0ppm/hem}$) until the end of the observation period. After 30 minutes hemorrhage was induced as described above and shed blood was retransfused after 60 min, all variables were recorded for another 60 min.

Infused CO

In a second series, the animals received either a CO-saturated saline solution ($intrav_{CO/hem}$) or saline ($intrav_{saline/hem}$) over 10 min, followed by hemorrhage and retransfusion of the shed blood, as detailed above.

In a control series, animals received either CO-saline ($intrav_{CO}$) or saline ($intrav_{saline}$) without any further intervention to exclude time related effects.

At the end of each intervention, blood samples were obtained for blood gas analysis.

Statistical analysis

Data for analysis were obtained during the last five minutes of each intervention under steady state conditions. Normal

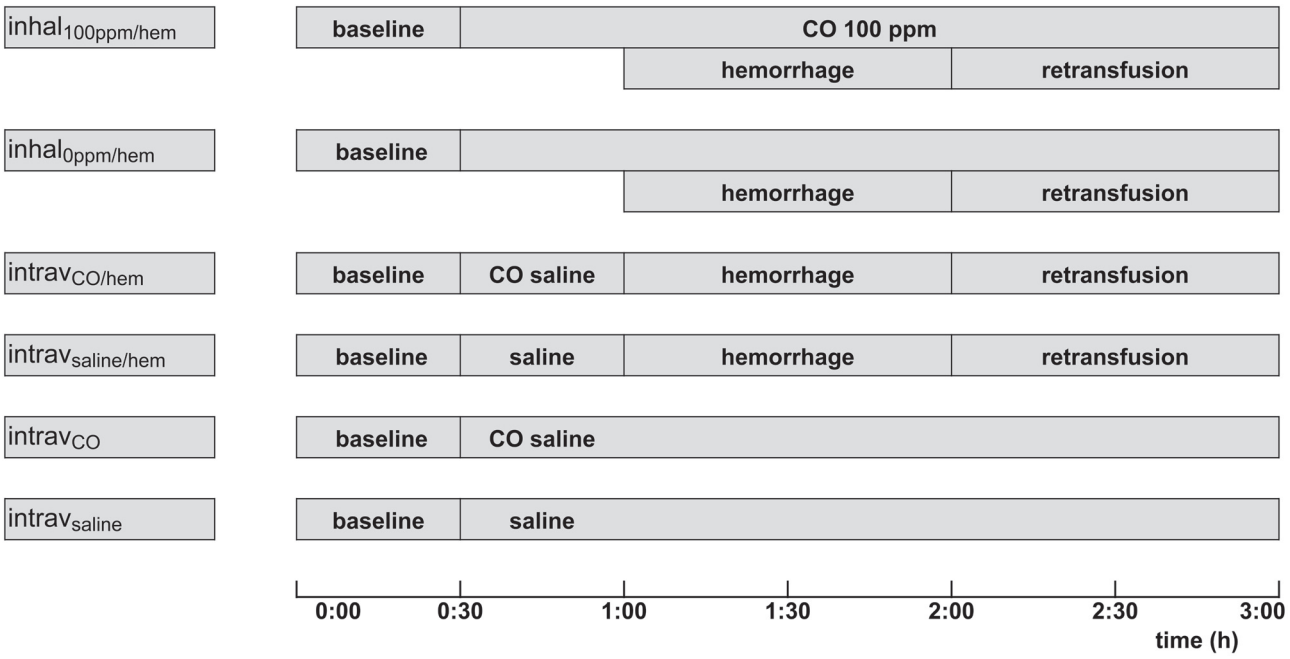

Figure 1. Experimental protocol. Interventions of all groups over time course from 0:00 to 03:00 hours. After baseline recordings for 30 minutes, the animals were continuously ventilated either with 100 ppm CO (group $inhal_{100ppm/hem}$) or without (group $inhal_{0ppm/hem}$), followed by hemorrhage after 1 hour and retransfusion of the shed blood after 2 hours. In the following groups, after baseline recordings the animals received either a CO-saturated saline solution 'CO-saline' (group $intrav_{CO/hem}$) or saline (group $intrav_{saline/hem}$) over 10 min, followed by hemorrhage and retransfusion, as detailed above. In a control series, animals received either CO-saline (group $intrav_{CO}$) or saline (group $intrav_{saline}$) without any further intervention.

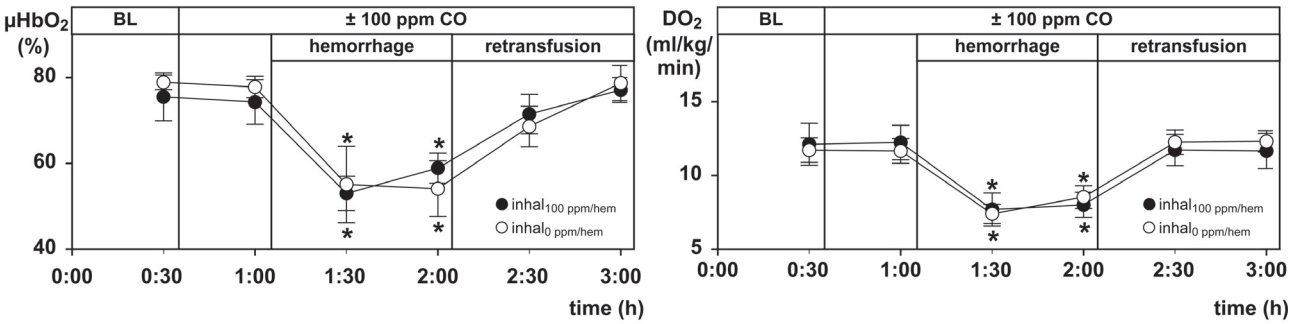

Figure 2. Microvascular hemoglobin saturation (μHbO_2) and systemic oxygen delivery (DO_2) in the course of time under baseline conditions (BL), under continuous ventilation either with 100 ppm carbon monoxide (closed circle) or without (open circle) during hemorrhage and retransfusion. Data presented as absolute values, mean ± SD, $n = 6$; * $p < 0.05$ *vs.* BL.

data distribution was confirmed by the Kolmogorov-Smirnov test (StatView V4.1, SAS-Institute Inc., Cary, NC, USA). All data are presented as absolute values of mean ± standard deviation (mean ± SD) for $n = 6$ animals *per* group in the first series and $n = 5$ in the second series, because one animal died before completion of all experiments. Differences within the groups were tested using an analysis of variance for repeated measurements (ANOVA) and a Fisher's PLSD test as post hoc test (StatView V4.1, SASInstituteInc, Cary, NC, USA). $p < 0.05$ was considered significant.

Results

Both inhalative and intravenous application of CO increased carboxyhemoglobin. This, however, did not alter microvascular hemoglobin oxygenation of the mucosa neither under physiological conditions nor after hemorrhage.

Inhalative protocol

Inhalation of air containing 100 ppm CO increased CO-Hb from 5.4 ± 0.6 to 6.3 ± 1.0% after 30 min and finally to 9.6 ± 1.0% at the end of the observation period, whereas CO-concentration remained unchanged during time control experiments without carbon monoxide ($inhal_{0ppm/hem}$). This, however, had no effect on μHbO_2 which remained unchanged and almost identical in both groups (Fig. 2). During hemorrhage, μHbO_2 decreased in both groups without significant differences between the groups, consistent with a decrease of oxygen delinery (DO_2) and was restored after retransfusion of the shed blood. Further variables are shown in Table 1 and 2.

Intravenous protocol

Infusion of a CO-saturated solution increased CO-concentration from 5.6 ± 0.6 to 6.4 ± 0.7% ($intrav_{CO}$) and from 4.8 ± 0.1 to 5.9 ± 0.1% ($intrav_{CO/hem}$) without any further

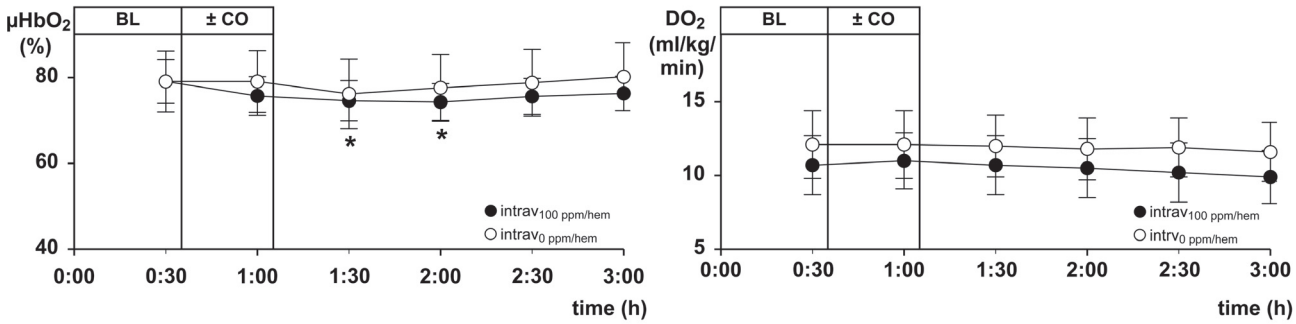

Figure 3. Microvascular hemoglobin saturation (μHbO_2) and systemic oxygen delivery (DO_2) in the course of time under baseline conditions (BL) followed by administration of saturated CO-solution (closed circle) or saline alone (open circle). Data presented as absolute values, mean ± SD, $n = 5$; * $p < 0.05$ *vs.* BL.

changes over time. At the end of the observation period maximum CO-Hb levels were lower after intravenous than inhalative application. During control experiments ($intrav_{saline}$/$intrav_{saline/hem}$) CO-Hb remained unchanged. Again, this did not have an effect on μHbO_2 in any group. μHbO_2 decreased during hypovolemia, increased after retransfusion to baseline values and did not change in both groups without hemorrhage (Fig. 3 and 4). The decrease during hemorrhagic shock is related to the decrease of DO_2, which is due to a decreased cardiac output. The increase of CO-Hb did not show any influence on μHbO_2. Further variables are shown in Table 1 and 2.

Table 1. Microvascular oxygenation and hemodynamic variables

Variable	Group	Baseline	CO	Hemorrhage 1	Hemorrhage 2	Retransfusion 1	Retransfusion 2
μHbO_2	$intrav_{CO}$	79 ± 5	76 ± 4	75 ± 5*	74 ± 4*	76 ± 4	76 ± 4
	$intrav_{saline}$	79 ± 7	79 ± 7	76 ± 8	78 ± 8	79 ± 8	80 ± 8
	$intrav_{CO/hem}$	74 ± 4	77 ± 4	53 ± 6*	55 ± 5*	72 ± 7	77 ± 5
	$intrav_{saline/hem}$	78 ± 3	79 ± 3	59 ± 9*	58 ± 8*	75 ± 5	78 ± 7
	$inhal_{100ppm/hem}$	76 ± 6	74 ± 5	53 ± 4*	59 ± 4*	72 ± 5	77 ± 3
	$inhal_{0ppm/hem}$	79 ± 2	78 ± 3	55 ± 9*	54 ± 7*	69 ± 5	79 ± 4
DO_2 (ml/kg/min)	$intrav_{CO}$	11 ± 2	11 ± 2	11 ± 2	11 ± 2	10 ± 2	10 ± 2*
	$intrav_{saline}$	12 ± 2	12 ± 2	12 ± 2	12 ± 2	12 ± 2	12 ± 2
	$intrav_{CO/hem}$	11 ± 1	11 ± 1	7 ± 1*	8 ± 1*	11 ± 1	11 ± 1
	$intrav_{saline/hem}$	12 ± 1	12 ± 1	9 ± 1*	9 ± 1*	12 ± 1	12 ± 1
	$inhal_{100ppm/hem}$	12 ± 1	12 ± 1	8 ± 1*	8 ± 1*	12 ± 1	12 ± 1
	$inhal_{0ppm/hem}$	12 ± 1	12 ± 1	7 ± 1*	9 ± 1*	12 ± 1	12 ± 1*
Cardiac output (ml/kg/min)	$intrav_{CO}$	75 ± 9	81 ± 10*	78 ± 10	80 ± 9*	77 ± 9	75 ± 8
	$intrav_{saline}$	75 ± 9	79 ± 9	77 ± 8	77 ± 7	77 ± 6	75 ± 7
	$intrav_{CO/hem}$	74 ± 5	79 ± 6	53 ± 4*	58 ± 4*	79 ± 5	79 ± 6
	$intrav_{saline/hem}$	77 ± 7	84 ± 7	58 ± 6*	61 ± 4*	84 ± 6	82 ± 5
	$inhal_{100ppm/hem}$	87 ± 8	87 ± 7	55 ± 6*	59 ± 5*	86 ± 6	86 ± 6
	$inhal_{0ppm/hem}$	83 ± 7	82 ± 6	52 ± 3*	60 ± 4*	85 ± 5	86 ± 6
SVR (mmHg·min/l)	$intrav_{CO}$	32 ± 5	28 ± 4*	30 ± 4*	30 ± 4*	31 ± 4	31 ± 4
	$intrav_{saline}$	31 ± 3	28 ± 2*	30 ± 2	31 ± 2	30 ± 2	31 ± 2
	$intrav_{CO/hem}$	30 ± 2	27 ± 2*	34 ± 2*	34 ± 2*	31 ± 1	29 ± 1
	$intrav_{saline/hem}$	29 ± 2	26 ± 2	33 ± 2*	33 ± 1*	31 ± 1	29 ± 1
	$inhal_{100ppm/hem}$	27 ± 2	27 ± 2	33 ± 2*	36 ± 2*	31 ± 1*	29 ± 1
	$inhal_{0ppm/hem}$	27 ± 2	27 ± 1	34 ± 2*	35 ± 2*	32 ± 1*	28 ± 1
MAP (mmHg)	$intrav_{CO}$	62 ± 3	59 ± 3*	61 ± 3	62 ± 3	62 ± 3	61 ± 3
	$intrav_{saline}$	62 ± 3	61 ± 3*	62 ± 3	64 ± 3*	64 ± 3*	64 ± 3*
	$intrav_{CO/hem}$	61 ± 2	59 ± 2	49 ± 2*	54 ± 3*	68 ± 3*	64 ± 3
	$intrav_{saline/hem}$	61 ± 1	60 ± 1	51 ± 2*	56 ± 2*	71 ± 4	66 ± 3
	$inhal_{100ppm/hem}$	62 ± 1	63 ± 1	49 ± 3*	57 ± 3	72 ± 3*	66 ± 2*
	$inhal_{0ppm/hem}$	60 ± 1	61 ± 1	49 ± 2*	56 ± 3	75 ± 2*	66 ± 1*
HR (1/min)	$intrav_{CO}$	108 ± 8	104 ± 6	103 ± 7*	102 ± 6*	101 ± 6*	98 ± 6*
	$intrav_{saline}$	108 ± 8	105 ± 7	104 ± 7*	103 ± 7*	102 ± 7*	100 ± 6*
	$intrav_{CO/hem}$	106 ± 4	103 ± 4	99 ± 4*	103 ± 5	98 ± 4*	95 ± 4*
	$intrav_{saline/hem}$	107 ± 5	105 ± 4	104 ± 4	106 ± 4	100 ± 4	97 ± 5*
	$inhal_{100ppm/hem}$	110 ± 6	109 ± 5	104 ± 4	109 ± 5	104 ± 5*	101 ± 6*
	$inhal_{0ppm/hem}$	111 ± 6	110 ± 6	111 ± 4	114 ± 5	107 ± 5	103 ± 6*

Effects of inhaled or infused carbon monoxide under hemorrhage (for 30 min "Hemorrhage 1" and 60 minutes "Hemorrhage 2") and retransfusion (after 30 min "Retransfusion 1" and 60 min "Retranfusion 2") on gastric microvascular mucosal oxygen saturation (μHbO_2), oxygen delivery (DO_2), cardiac output, systemic vascular resistance (SVR), mean arterial pressure (MAP) and heart rate (HR). Data presented as absolute values, $n = 6$ *per* group for inhaled CO and $n = 5$ *per* group for infused CO, mean ± SD; * $p < 0.05$ *versus* baseline.

Discussion

Critique of methods

Repetitive experiments were performed on six healthy, chronically instrumented dogs in randomized order. As each dog underwent each of the protocols it served as its own control minimizing interindividual differences. Between two experiments with the same animal an interval of at least two weeks was guaranteed to ensure complete recovery of the animals and to prevent carryover effects.

Carbon monoxide

Carbon monoxide induces acute and chronic toxicity in high doses with increased CO-Hb but is also produced endogenously with a physiological role as a signalling molecule (Boehning and Snyder 2003), exerting protective and vasoactive effects (Bauer and Pannen 2009). Those protective effects can be triggered by low doses of exogenous CO that do not increase CO-Hb to toxic levels and are known to be dose-dependent (Hangai-Hoger 2007). Therefore, we tested the concentration of infused CO on gastric circulation known to have the greatest

Table 2. Blood gas tensions, pH and total hemoglobin

Variable	Group	Baseline	CO	Hemorrhage 1	Hemorrhage 2	Retransfusion 1	Retransfusion 2
CO-Hb (%)	$intrav_{CO}$	5.6 ± 0.6	6.4 ± 0.7*	6.6 ± 0.7*	6.4 ± 0.8*	6.5 ± 0.6*	6.6 ± 0.8*
	$intrav_{saline}$	4.5 ± 0.1	4.7 ± 0.1	4.8 ± 0.2*	4.7 ± 0.1	5.0 ± 0.1*	5.0 ± 0.1*
	$intrav_{CO/hem}$	4.8 ± 0.1	5.9 ± 0.1*	5.4 ± 0.1*	5.3 ± 0.1*	5.8 ± 0.1*	5.8 ± 0.1*
	$intrav_{saline/hem}$	4.9 ± 0.2	4.9 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	5.2 ± 0.2*	5.1 ± 0.2
	$inhal_{100ppm/hem}$	5.4 ± 0.6	6.3 ± 1.0	6.6 ± 1.0	7.5 ± 1.0*	8.8 ± 1.0*	9.6 ± 1.0*
	$inhal_{0ppm/hem}$	6.7 ± 0.8	7.2 ± 1.0	6.8 ± 0.9	6.4 ± 0.8	6.3 ± 0.7	6.3 ± 0.7
pO_2 (mmHg)	$intrav_{CO}$	107 ± 9	111 ± 10	112 ± 11	113 ± 11	109 ± 9	109 ± 9
	$intrav_{saline}$	110 ± 10	113 ± 9	109 ± 7	115 ± 8	113 ± 10	116 ± 10
	$intrav_{CO/hem}$	107 ± 9	116 ± 9	112 ± 11	108 ± 11	118 ± 10*	116 ± 10
	$intrav_{saline/hem}$	100 ± 8	107 ± 7*	99 ± 6*	105 ± 9	111 ± 7*	111 ± 8*
	$inhal_{100ppm/hem}$	97 ± 10	100 ± 10	99 ± 9	99 ± 9	107 ± 10*	105 ± 10*
	$inhal_{0ppm/hem}$	101 ± 8	102 ± 6	93 ± 4	106 ± 9	106 ± 7	106 ± 6
pCO_2 (mmHg)	$intrav_{CO}$	37 ± 1	38 ± 1	37 ± 1	38 ± 1	39 ± 2	38 ± 2
	$intrav_{saline}$	38 ± 2	37 ± 1	37 ± 1	38 ± 1	38 ± 1	38 ± 2
	$intrav_{CO/hem}$	38 ± 1	38 ± 1	42 ± 1*	40 ± 2*	41 ± 1*	41 ± 2*
	$intrav_{saline/hem}$	37 ± 1	36 ± 1	40 ± 1*	39 ± 1*	38 ± 1	39 ± 1*
	$inhal_{100ppm/hem}$	36 ± 1	37 ± 1*	39 ± 1*	39 ± 1*	37 ± 1	37 ± 1
	$inhal_{0ppm/hem}$	36 ± 1	36 ± 1	38 ± 1	38 ± 1	37 ± 1	37 ± 1*
pH	$intrav_{CO}$	7.35 ± 0.02	7.34 ± 0.02	7.35 ± 0.02	7.34 ± 0.02	7.34 ± 0.02	7.34 ± 0.02
	$intrav_{saline}$	7.35 ± 0.03	7.34 ± 0.03	7.35 ± 0.02	7.35 ± 0.02	7.34 ± 0.02*	7.34 ± 0.03*
	$intrav_{CO/hem}$	7.35 ± 0.01	7.35 ± 0.01	7.29 ± 0.02*	7.30 ± 0.02*	7.32 ± 0.01*	7.33 ± 0.01*
	$intrav_{saline/hem}$	7.37 ± 0.01	7.36 ± 0.01	7.31 ± 0.01*	7.32 ± 0.02*	7.34 ± 0.01*	7.35 ± 0.01*
	$inhal_{100ppm/hem}$	7.38 ± 0.01	7.37 ± 0.01*	7.31 ± 0.01*	7.32 ± 0.01*	7.35 ± 0.01*	7.36 ± 0.01
	$inhal_{0ppm/hem}$	7.38 ± 0.01	7.36 ± 0.01	7.31 ± 0.01*	7.33 ± 0.02*	7.35 ± 0.01*	7.36 ± 0.01*
ctHb (g/dl)	$intrav_{CO}$	11.4 ± 1.0	11.0 ± 0.9	11.0 ± 1.0	10.6 ± 1.1*	10.7 ± 1.2	10.6 ± 1.2*
	$intrav_{saline}$	12.9 ± 0.8	12.2 ± 0.9	12.3 ± 1.0	12.3 ± 0.9	12.3 ± 1.1	12.3 ± 1.0
	$intrav_{CO/hem}$	11.8 ± 0.7	11.1 ± 0.7*	11.3 ± 0.6*	11.1 ± 0.6*	11.1 ± 0.7*	11.1 ± 0.7*
	$intrav_{saline/hem}$	12.4 ± 0.8	11.6 ± 0.7*	11.5 ± 0.8*	11.6 ± 0.7*	11.6 ± 0.7*	11.7 ± 0.7*
	$inhal_{100ppm/hem}$	11.6 ± 0.7	11.7 ± 0.4	11.7 ± 0.3	11.6 ± 0.2	11.5 ± 0.3	11.6 ± 0.3
	$inhal_{0ppm/hem}$	11.9 ± 1.0	11.9 ± 0.7	12.0 ± 0.9	11.9 ± 0.8	11.9 ± 0.7	11.9 ± 0.8

Effects of inhaled or infused carbon monoxide under hemorrhage (for 30 minutes "Hemorrhage 1" and 60 minutes "Hemorrhage 2") and retransfusion (after 30 minutes "Retransfusion 1" and 60 minutes "Retranfusion 2") on carboxyhemoglobin (CO-Hb), arterial oxygen tension (pO_2), arterial carbon dioxide tension (pCO_2), pH and total hemoglobin (ctHb); data presented as absolute values, $n = 6$ *per* group for inhaled CO and $n = 5$ *per* group for infused CO, mean ± SD; * $p < 0.05$ *versus* baseline.

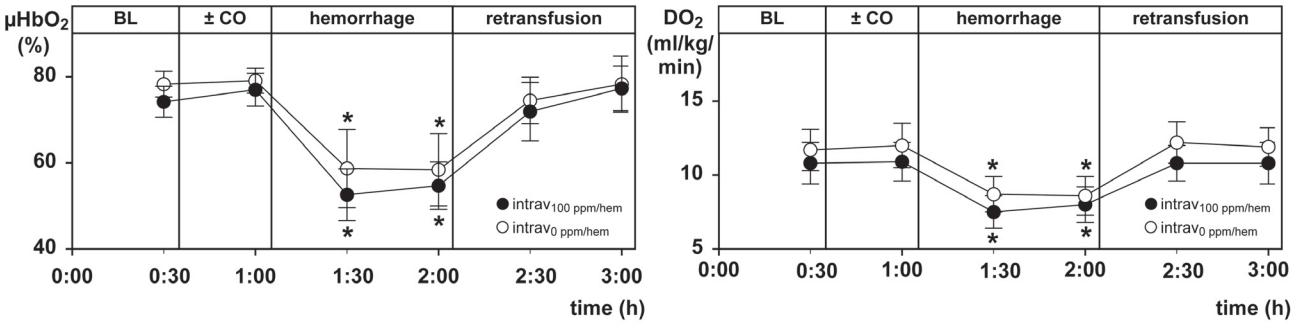

Figure 4. Microvascular hemoglobin saturation (μHbO_2) and systemic oxygen delivery (DO_2) in the course of time under baseline conditions (BL), under administration of saturated CO-solution (closed circle) or saline alone (open circle) followed by hemorrhage and retransfusion. Data presented as absolute values, mean ± SD, $n = 5$; * $p < 0.05$ *vs.* BL.

impact on circulation in other tissues (Hangai-Hoger 2007) with little effect on CO-Hb. Recent studies have shown that low doses of CO affect microcirculation even without increasing CO-Hb (Guo et al. 2004; Hangai-Hoger 2007).

The low dose of inhaled CO used in this study has been shown to have cytoprotective and anti-inflammatory effects in previous studies (Bathoorn et al. 2007) whereby the mechanisms are largely unknown but will be discussed below. The aim was to analyze whether those cytoprotective concentrations of CO induce macro- or microcirculatory disturbances regarding systemic or local oxygenation in the gastric mucosa.

Thus, the low dose of CO was sufficient to increase CO-Hb only to a small amount similar to intravenous application without inducing toxic effect and was therefore adequate to analyze effects on microcirculatory circulation and oxygenation.

Though, long-term effects of CO as a modulator of heme biosynthesis and as an oxygen sensor (Dubin 2007) on the gastric oxygenation cannot be analyzed in this short observation period. Additionally, CO can induce suspended animation, a phenomenon similar to hibernation, and could improve survival during hypoxia and sepsis (Kajimura et al. 2010). This phenomenon cannot be observed in this short period. Yet, our hemorrhagic shock model is adequate to observe short-term vasoactive effects of CO, especially under hemorrhagic conditions and during therapeutic blood transfusion. Nevertheless, we analyzed one dose and cannot rule out the possible usefulness of CO at other doses.

Interpretation of the results

In contrast to human beings the animals started off at quite high CO-Hb values, however, these values are common in dogs and similar CO-Hb values in dogs have been reported elsewhere previously (Wack 2005).

CO under physiological conditions

Both inhaled and infused CO increased CO-Hb only to a small amount without effect on local or systemic oxygenation. Therefore, presumed cytoprotective effects of low dose CO can be further investigated and applied without compromising microvascular oxygenation. The only small increase of CO-Hb might be due to self-inhibition. CO is known to autoinhibit HO and thus its own synthesizing enzyme. While endogenous CO does not inhibit HO reaction (Marks et al. 2002), larger amounts of exogenous CO attenuated this reaction (Yoshida et al. 1980).

Nevertheless, the reported vasodilatory effects of other studies (Hartsfield et al. 2004; Nishikawa et al. 2004; Kanu et al. 2006; Hosgood et al. 2008) could not be observed in this experiment. One reason might be that some of these studies analyzed isolated organs (Hosgood et al. 2008) and the shown effects might not become evident in a whole organism. Other *in vivo* animal studies did not use application of exogenous CO but instead indirect endogenous generation *via* HO-1 stimulation (Hartsfield et al. 2004; Nishikawa et al. 2004; Kanu et al. 2006). These reported HO-mediated vasodilatory effects might not be CO-related or demonstrate a difference between endogenous and exogenous effects of CO on the μHbO_2. Another difference to invasive measurements (Nishikawa et al. 2004; Hangai-Hoger 2007) is our non-traumatic approach which enables measurement under physiological conditions. This is important because surgical manipulation alters blood flow (Gelman 1976). Though we used equal concentrations to previous studies showing vasodilatory effects, we still do not know the local tissue concentration of CO in gastric vasculature. CO has to cross the membranes by diffusion, although the existence of transport is discussed. Thus, the diffusion and hence intracellular concentration depends on the above mentioned conditions of the local environment which might be different in gastric mucosa compared to the hamster window chamber model and other studies. Especially for gastric

gland cells it has been shown that their permeability for gaseous molecules is extremely reduced (Waisbren et al. 1994). However, this has not been shown for CO and gastric gland cells are not comparable to gastric vasculature. Differences in local tissue concentrations of CO might be an explanation for our diverging results, especially since vasodilatory effects have been shown to be concentration-dependent (Hangai-Hoger 2007). Still, at the moment there is no sufficient method to estimate intracellular CO-concentration, e. g. in the gastric mucosa, *in vivo*.

Furthermore CO might regulate vascular tone mainly by modulation of the generation of NO, and its actions seem to depend in part on colocalization of the NO-synthase and HO with sCG (Kajimura et al. 2010). Colocalization might be different in the hamster window chamber model and explain different effects of CO on different tissues.

Another likely reason for our divergent results is the different approach in our study, which is the first to analyze microvascular oxygenation rather than perfusion.

The reported CO related improvement of survival after gut ischemia (Nakao et al. 2003) and in septic animals (Chung et al. 2008) is – according to our results – not related to an improved oxygenation and maintained gut barrier but rather to long-term, immunomodulatory effects of CO (Bauer and Pannen 2009) as well as enhanced colonic cell restitution. These anti-inflammatory effects are reduction in myeloperoxidase activity, reduced production of keratinocyte chemoattractant and tumor necrosis factor-α along with inhibited nuclear translocation of NF-κB (Takagi et al. 2010). Enhanced colonic epithelial cell restitution after CO treatment to myofibroblasts is related to increased fibroblast growth factor 15 expression *via* inhibition of microRNA miR-710 (Uchiyama et al. 2009).

CO under hemorrhagic conditions

Especially during hypovolemia CO might play a crucial role, as it is known to reduce organ damage under hemorrhage (Sakai et al. 2009). Furthermore, under stress conditions enhanced production of CO due to induction of HO could be observed (Maines 1997). Under hemorrhage this might be important in the neurotransmission of the enteric nervous system (Boehning and Snyder 2003) and especially in gastrointestinal vessels (Zakhary et al. 1996).

Nevertheless, even during hemorrhagic shock CO did not alter microvascular oxygenation in our study. The expected effects might be important in the long term, i.e. by enzyme induction, but could not affect oxygenation under hemorrhage in this short observation period. Still, our results suggest that cytoprotective and immunomodulatory effects can be used in hemorrhagic shock without compromising local oxygenation. This oxygenation is important for the maintenance of the gut barrier function (Trzeciak et al. 2007, 2008), which is most probably not affected by CO.

Conclusions

In summary, despite the previously reported increase of perfusion in different tissues triggered by CO, the same dose does not affect gastric microcirculation at the same time.

References

Aono S., Honma Y., Ohkubo K., Tawara T., Kamiya T., Nakajima H. (2000): CO sensing and regulation of gene expression by the transcriptional activator CooA. J. Inorg. Biochem. **82,** 51–56 http://dx.doi.org/10.1016/S0162-0134(00)00139-2

Barker S. J., Tremper K. K. (1987): The effect of carbon monoxide inhalation on pulse oximetry and transcutaneous PO2. Anesthesiology **66,** 677–679 http://dx.doi.org/10.1097/00000542-198705000-00014

Bathoorn E., Slebos D. J., Postma D. S., Koeter G. H., van Oosterhout A. J., van der Toorn M., et al. (2007): Anti-inflammatory effects of inhaled carbon monoxide in patients with COPD: a pilot study. Eur. Respir. J. **30,** 1131–1137 http://dx.doi.org/10.1183/09031936.00163206

Bauer I., Pannen B. H. (2009): Bench-to-bedside review: Carbon monoxide--from mitochondrial poisoning to therapeutic use. Crit. Care **13,** 220 http://dx.doi.org/10.1186/cc7887

Bellamy M. C., Mullane D., O'Beirne H. A., Young Y., Pollard S. G., Lodge J. P. (1997): Dopexamine and microcirculatory flow in transplanted small bowel: the Leeds experience. Transplant. Proc. **29,** 1847–1849 http://dx.doi.org/10.1016/S0041-1345(97)00093-6

Berk P. D., Rodkey F. L., Blaschke T. F., Collison H. A., Waggoner J. G. (1974): Comparison of plasma bilirubin turnover and carbon monoxide production in man. J. Lab. Clin. Med. **83,** 29–37

Boehning D., Snyder S. H. (2003): Novel neural modulators. Annu. Rev. Neurosci. **26,** 105-131 http://dx.doi.org/10.1146/annurev.neuro.26.041002.131047

Chen L. W., Egan L., Li Z. W., Greten F. R., Kagnoff M. F., Karin M. (2003): The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. Nat. Med. **9,** 575–581 http://dx.doi.org/10.1038/nm849

Chung S. W., Liu X., Macias A. A., Baron R. M., Perrella M. A. (2008): Heme oxygenase-1-derived carbon monoxide enhances the host defense response to microbial sepsis in mice. J. Clin. Invest. **118,** 239–247 http://dx.doi.org/10.1172/JCI32730

Deitch E. A., Forsythe R., Anjaria D., Livingston D. H., Lu Q., Xu D. Z., et al. (2004): The role of lymph factors in lung injury, bone marrow suppression, and endothelial cell dysfunction

in a primate model of trauma-hemorrhagic shock. Shock **22,** 221–228
http://dx.doi.org/10.1097/01.shk.0000133592.55400.83

Dubin A. (2007): Carbon monoxide: venom, endogenous mediator, or therapeutic agent? Crit. Care Med. **35,** 1213–1214
http://dx.doi.org/10.1097/01.CCM.0000259170.25548.56

Edouard A. R., Degremont A. C., Duranteau J., Pussard E., Berdeaux A., Samii K. (1994): Heterogeneous regional vascular responses to simulated transient hypovolemia in man. Intensive Care Med. **20,** 414–420
http://dx.doi.org/10.1007/BF01710651

Engel R. R., Matsen J. M., Chapman S. S., Schwartz S. (1972): Carbon monoxide production from heme compounds by bacteria. J. Bacteriol. **112,** 1310–1315

Fournell A., Schwarte L. A., Kindgen-Milles D., Muller E., Scheeren T. W. (2003): Assessment of microvascular oxygen saturation in gastric mucosa in volunteers breathing continuous positive airway pressure. Crit. Care Med. **31,** 1705–1710
http://dx.doi.org/10.1097/01.CCM.0000063281.47070.53

Frank K. H., Kessler M., Appelbaum K., Dummler W. (1989): The Erlangen micro-lightguide spectrophotometer EMPHO I. Phys. Med. Biol. **34,** 1883–1900
http://dx.doi.org/10.1088/0031-9155/34/12/011

Gandjbakhche A. H., Bonner R. F., Arai A. E., Balaban R. S. (1999): Visible-light photon migration through myocardium in vivo. Am. J. Physiol. **277,** H698–704

Gelman S. I. (1976): Disturbances in hepatic blood flow during anesthesia and surgery. Arch. Surg. **111,** 881–883
http://dx.doi.org/10.1001/archsurg.1976.01360260049012

Guo Y., Stein A. B., Wu W. J., Tan W., Zhu X., Li Q. H., et al. (2004): Administration of a CO-releasing molecule at the time of reperfusion reduces infarct size in vivo. Am. J. Physiol. Heart Circ. Physiol. **286,** H1649–1653
http://dx.doi.org/10.1152/ajpheart.00971.2003

Gutierrez G., Rotman H. H., Reid C. M., Dantzker D. R. (1985): Comparison of canine cardiovascular response to inhaled and intraperitoneally infused CO. J. Appl. Physiol. **58,** 558–563

Hangai-Hoger (2007). Microvascular and systemic effects following top load administration of saturated carbon monoxide-saline solution. Crit. Care Med. **35,** 1123–1132
http://dx.doi.org/10.1097/01.CCM.0000259533.84180.C7

Hartsfield C. L., McMurtry I. F., Ivy D. D., Morris K. G., Vidmar S., Rodman D. M., et al. (2004): Cardioprotective and vasomotor effects of HO activity during acute and chronic hypoxia. Am. J. Physiol. Heart Circ. Physiol. **287,** H2009–2015
http://dx.doi.org/10.1152/ajpheart.00394.2002

Hosgood S. A., Bagul A., Kaushik M., Rimoldi J., Gadepalli R. S., Nicholson M. L. (2008): Application of nitric oxide and carbon monoxide in a model of renal preservation. Br. J. Surg. **95,** 1060–1067
http://dx.doi.org/10.1002/bjs.6174

Jakob S. M., Takala J. (2000): Gut perfusion in the critically ill. Intensive Care Med. **26,** 813-815
http://dx.doi.org/10.1007/s001340051253

Kajimura M., Shimoyama M., Tsuyama S., Suzuki T., Kozaki S., Takenaka S., et al. (2003): Visualization of gaseous monoxide reception by soluble guanylate cyclase in the rat retina. FASEB. J. **17,** 506–508

Kajimura M., Fukuda R., Bateman R. M., Yamamoto T., Suematsu M. (2010): Interactions of multiple gas-transducing systems: hallmarks and uncertainties of CO, NO, and H2S gas biology. Antioxid. Redox. Signal. **13,** 157–192
http://dx.doi.org/10.1089/ars.2009.2657

Kanten W. E., Penney D. G., Francisco K., Thill J. E. (1983): Hemodynamic responses to acute carboxyhemoglobinemia in the rat. Am. J. Physiol. **244,** H320–327

Kanu A., Whitfield J., Leffler C. W. (2006): Carbon monoxide contributes to hypotension-induced cerebrovascular vasodilation in piglets. Am. J. Physiol. Heart Circ. Physiol. **291,** H2409–2414
http://dx.doi.org/10.1152/ajpheart.01368.2005

Kazama T., Ikeda K. (1988): Comparison of MAC and the rate of rise of alveolar concentration of sevoflurane with halothane and isoflurane in the dog. Anesthesiology **68,** 435–437
http://dx.doi.org/10.1097/00000542-198803000-00020

Krug A. (2006): Microcirculation and oxygen supply of tissue: method of so-called 02C. Phlebologie **35,** 300–312 (in German)

Kuchenreuther S., Adler J., Schutz W., Eichelbronner O., Georgieff M. (1996): The erlanger microlightguide photometer: a new concept for monitoring intracapillary oxygen supply of tissue--first results and a review of the physiological basis. J. Clin. Monit. **12,** 211–224
http://dx.doi.org/10.1007/BF00857642

Leung F. W., Morishita T., Livingston E. H., Reedy T., Guth P. H. (1987): Reflectance spectrophotometry for the assessment of gastroduodenal mucosal perfusion. Am. J. Physiol. **252,** G797–804

Machens H. G., Pallua N., Mailaender P., Pasel J., Frank K. H., Reimer R., et al. (1995): Measurements of tissue blood flow by the hydrogen clearance technique (HCT): a comparative study including laser Doppler flowmetry (LDF) and the Erlangen micro-lightguide spectrophotometer (EMPHO). Microsurgery **16,** 808–817
http://dx.doi.org/10.1002/micr.1920161208

Maines M. D. (1997): The heme oxygenase system: a regulator of second messenger gases. Annu. Rev. Pharmacol. Toxicol. **37,** 517–554
http://dx.doi.org/10.1146/annurev.pharmtox.37.1.517

Marks G. S., Vreman H. J., McLaughlin B. E., Brien J. F., Nakatsu K. (2002): Measurement of endogenous carbon monoxide formation in biological systems. Antioxid. Redox. Signal. **4,** 271–277
http://dx.doi.org/10.1089/152308602753666325

Moore B. A., Overhaus M., Whitcomb J., Ifedigbo E., Choi A. M., Otterbein L. E., et al. (2005): Brief inhalation of low-dose carbon monoxide protects rodents and swine from postoperative ileus. Crit. Care Med. **33,** 1317–1326
http://dx.doi.org/10.1097/01.CCM.0000166349.76514.40

Nakao A., Kimizuka K., Stolz D. B., Neto J. S., Kaizu T., Choi A. M., et al. (2003): Carbon monoxide inhalation protects rat intestinal grafts from ischemia/reperfusion injury. Am. J. Pathol. **163,** 1587–1598
http://dx.doi.org/10.1016/S0002-9440(10)63515-8

Nishikawa Y., Stepp D. W., Merkus D., Jones D., Chilian W. M. (2004): In vivo role of heme oxygenase in ischemic coronary vasodilation. Am J Physiol Heart Circ Physiol. **286,** H2296–2304

http://dx.doi.org/10.1152/ajpheart.00671.2003
Pannen B. H., Kohler N., Hole B., Bauer M., Clemens M. G., Geiger K. K. (1998): Protective role of endogenous carbon monoxide in hepatic microcirculatory dysfunction after hemorrhagic shock in rats. J. Clin. Invest. **102,** 1220–1228
http://dx.doi.org/10.1172/JCI3428
Russell D. H., Barreto J. C., Klemm K., Miller T. A. (1995): Hemorrhagic shock increases gut macromolecular permeability in the rat. Shock **4,** 50–55
http://dx.doi.org/10.1097/00024382-199507000-00008
Sakai H., Horinouchi H., Tsuchida E., Kobayashi K. (2009): Hemoglobin vesicles and red blood cells as carriers of carbon monoxide prior to oxygen for resuscitation after hemorrhagic shock in a rat model. Shock **31,** 507–514
http://dx.doi.org/10.1097/SHK.0b013e318188f83d
Sato N., Kawano S., Kamada T., Takeda M. (1986): Hemodynamics of the gastric mucosa and gastric ulceration in rats and in patients with gastric ulcer. Dig. Dis. Sci. **31,** S35–41
http://dx.doi.org/10.1007/BF01309321
Scheeren T. W., Schwarte L. A., Loer S. A., Picker O., Fournell A. (2002): Dopexamine but not dopamine increases gastric mucosal oxygenation during mechanical ventilation in dogs. Crit. Care Med. **30,** 881–887
http://dx.doi.org/10.1097/00003246-200204000-00028
Siegemund M., van Bommel J., Ince C. (1999): Assessment of regional tissue oxygenation. Intensive Care Med. **25,** 1044–1060
http://dx.doi.org/10.1007/s001340051011
Takagi T., Naito Y., Uchiyama K., Suzuki T., Hirata I., Mizushima K., et al. (2010): Carbon monoxide liberated from carbon monoxide-releasing molecule exerts an anti-inflammatory effect on dextran sulfate sodium-induced colitis in mice. Dig. Dis. Sci. **56,** 1663–1671
http://dx.doi.org/10.1007/s10620-010-1484-y
Temmesfeld-Wollbruck B., Szalay A., Mayer K., Olschewski H., Seeger W., Grimminger F. (1998): Abnormalities of gastric mucosal oxygenation in septic shock: partial responsiveness to dopexamine. Am. J. Respir. Crit. Care. Med. **157,** 1586–1592
Trzeciak S., Dellinger R. P., Parrillo J. E., Guglielmi M., Bajaj J., Abate N. L., et al. (2007): Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann. Emerg. Med. **49,** 88–98, 98 e1–2
Trzeciak S., McCoy J. V., Phillip Dellinger R., Arnold R. C., Rizzuto M., Abate N. L., et al. (2008): Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. Intensive Care Med. **34,** 2210–2217
http://dx.doi.org/10.1007/s00134-008-1193-6
Uchiyama K., Naito Y., Takagi T., Mizushima K., Hayashi N., Harusato A., et al. (2009): Carbon monoxide enhance colonic epithelial restitution via FGF15 derived from colonic myofibroblasts. Biochem. Biophys. Res. Commun. **391,** 1122–1126
http://dx.doi.org/10.1016/j.bbrc.2009.12.035
von Spiegel T., Wietasch G., Bursch J., Hoeft A. (1996): Cardiac output determination with transpulmonary thermodilution. An alternative to pulmonary catheterization? Anaesthesist. **45,** 1045–1050 (in German)
Vreman H. J., Wong R. J., Sanesi C. A., Dennery P. A., Stevenson D. K. (1998): Simultaneous production of carbon monoxide and thiobarbituric acid reactive substances in rat tissue preparations by an iron-ascorbate system. Can. J. Physiol. Pharmacol. **76,** 1057-1065
http://dx.doi.org/10.1139/y98-126
Wack J. (2005): Die Einflüsse auf die arteriovenöse Carboxyhämoglobin (COHb) – Differenz im Blut von chronisch instrumentierten Hunden im Vergleich zum Menschen. (Thesis)
Waisbren S. J., Geibel J. P., Modlin I. M., Boron W. F. (1994): Unusual permeability properties of gastric gland cells. Nature **368,** 332–335
http://dx.doi.org/10.1038/368332a0
Wang R., Wu L., Wang Z. (1997): The direct effect of carbon monoxide on KCa channels in vascular smooth muscle cells. Pflügers Arch. **434,** 285–291
http://dx.doi.org/10.1007/s004240050398
Yoshida T., Noguchi M., Kikuchi G. (1980): A new intermediate of heme degradation catalyzed by the heme oxygenase system. J. Biochem. **88,** 557–563
Zakhary R., Gaine S. P., Dinerman J. L., Ruat M., Flavahan N. A., Snyder S. H. (1996): Heme oxygenase 2: endothelial and neuronal localization and role in endothelium-dependent relaxation. Proc. Natl. Acad. Sci. U. S. A. **93,** 795–798
http://dx.doi.org/10.1073/pnas.93.2.795
Zijlstra W. G., Buursma A. (1987): Spectrophotometry of hemoglobin: a comparison of dog and man. Comp. Biochem. Physiol. B. **88,** 251–255
http://dx.doi.org/10.1016/0305-0491(87)90109-X
Zijlstra W. G., Buursma A., Meeuwsen-van der Roest W. P. (1991): Absorption spectra of human fetal and adult oxyhemoglobin, de-oxyhemoglobin, carboxyhemoglobin, and methemoglobin. Clin. Chem. **37,** 1633–1638

Received: August 18, 2011
Final version accepted: September 30, 2011