

Demonstration and quantification of the redistribution and oxidation of carbon monoxide in the human body by tracer analysis

Makoto Sawano*

Department of Emergency Medicine, Saitama Medical Center, Saitama Medical University, Kawagoe-shi, Saitama, Japan

*Correspondence to: Makoto Sawano, M.D., Ph.D., sawano@me.com.

orcid: 0000-0003-3433-6189

Abstract

Numerous studies have confirmed the role of endogenous carbon monoxide (CO) gas as a signal transmitter. However, CO is considered an intracellular transmitter, as no studies have demonstrated the redistribution of CO from the blood to tissue cells. Tracer analyses of $^{13}\text{CO}_2$ production following ^{13}CO gas inhalation demonstrated that CO is oxidized to carbon dioxide (CO_2) in the body and that CO oxidation does not occur in the circulation. However, these results could not clearly demonstrate the redistribution of CO, because oxidation may have occurred in the airway epithelium. The objective of this study, therefore, was to definitively demonstrate and quantify the redistribution and oxidation of CO using time-course analyses of CO and $^{13}\text{CO}_2$ production following ^{13}CO -hemoglobin infusion. The subject was infused with 0.45 L of ^{13}CO -saturated autologous blood. Exhaled gas was collected intermittently for 36 hours for measurement of minute volumes of CO/ CO_2 exhalation and determination of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio. $^{13}\text{CO}_2$ production significantly increased from 3 to 28 hours, peaking at 8 hours. Of the infused CO, 81% was exhaled as CO and 2.6% as $^{13}\text{CO}_2$. Identical time courses of $^{13}\text{CO}_2$ production following ^{13}CO -hemoglobin infusion and ^{13}CO inhalation refute the hypothesis that CO is oxidized in the airway epithelium and clearly demonstrate the redistribution of CO from the blood to the tissues. Quantitative analyses have revealed that 19% of CO in the circulating blood is redistributed to tissue cells, whereas 2.6% is oxidized there. Overall, these results suggest that CO functions as a systemic signal transmitter.

Key words: carbon monoxide; redistribution; oxidization; tracer analysis; stable isotope; signal transmitter, quantitative analysis; hemoglobin

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INTRODUCTION

Numerous studies have shown that endogenous carbon monoxide (CO) acts as a signal transmitter (Wu and Wang, 2005). However, CO is thought to act only intracellularly, as no studies have demonstrated redistribution of CO from the blood to tissues (Halperin et al., 1959; Coburn, 1970; Stewart, 1975; Fujimoto et al., 2004; Wang, 2004). Detection of $^{13}\text{CO}_2$ production following the inhalation of ^{13}CO gas demonstrates the oxidation of CO in human tissues (Sawano and Shimouchi, 2010). However, the possibility of CO oxidation in the airway epithelium precludes definitive demonstration of redistribution. The objective of

this study, therefore, was to demonstrate and quantify the redistribution and oxidation of CO in the human body by monitoring CO and $^{13}\text{CO}_2$ production following infusion of ^{13}CO -hemoglobin.

SUBJECTS AND METHODS

Ethics statement and subjects

The ethics committee of Saitama Medical University in Japan approved the experimental protocol of this study. The subject, who provided written informed consent before the experiment, was a healthy, non-smoking 50 years old male volunteer.

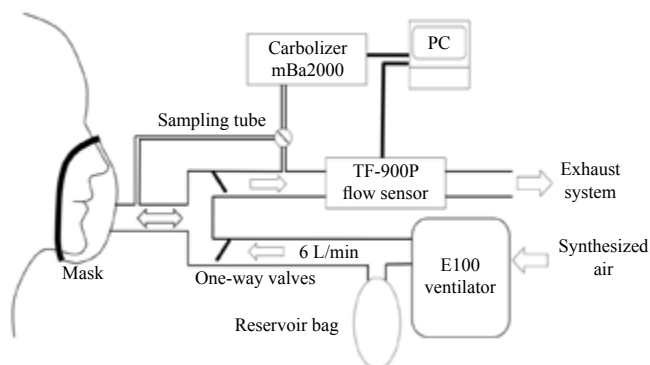


Figure 1: Schematic diagram of the device used to estimate the minute volumes of carbon monoxide and carbon dioxide exhalation each minute and the end-tidal breath carbon monoxide concentration with each breath.

Baseline exhaled gas measurements and sampling

Throughout the experiment, a physician closely observed the subject and monitored his electrocardiogram and pulse-oximetry to determine arterial carboxy- and oxy-hemoglobin fractions (FCO_{Hb} and FO₂Hb), and blood pressure. The experiment was conducted in an operating room equipped with a forced ventilation system. During the experiment, the subject inhaled synthesized air (21% oxygen gas and 79% nitrogen gas), except for limited interruptions.

At the beginning of the experiment, the subject held his breath for 20 seconds and then exhaled into a 1.3-L gas-sampling bag (pylori exhaled gas sampling bag, Fukuda Denshi Co., Ltd., Tokyo, Japan). The procedure was repeated every 10 minutes, until 25 bags of exhaled gas were collected as baseline gas samples. The subject was then asked to breathe freely for 5 minutes using a device consisting of a respiratory circuit, a ventilator (E100, Newport Medical Instruments Inc., Costa Mesa, CA, USA), a mask, a flow sensor (TF-900P, Nihon Kohden Corp., Tokyo, Japan), an electrochemical sensor (Carbolizer mBA-1000, Taiyo Co., Ltd., Osaka, Japan), and a gas circuit with two one-way valves (**Figure 1**). The E100 generates and regulates a constant 6 L/min flow of synthesized air. The Carbolizer electrochemical sensor is capable of determining the CO concentration in the outflow gas every second, at a resolution of 0.1 ppm (Sawano et al., 2006). We processed simultaneous outputs from the Carbolizer and the TF-900P flow sensor to estimate the minute volume of CO (MVCO) and CO₂ (MVCO₂) in exhaled gas, each minute. The device is also capable of determining the end-tidal breath CO (ETCO) concentration with each breath.

Infusion of ¹³CO-hemoglobin

One week prior to initiation of the experiment, we estimated the circulating blood volume of the subject using

the CO-hemoglobin dilution technique (Sawano et al., 2006). We drew 0.45 L (approximately 9% of the estimated circulating blood volume) of venous blood from the subject, sealed it in a sterilized plastic bag with 100 units of heparin and 0.4 L of 100% ¹³CO gas (Cambridge Isotope Laboratories, Inc., Andover, MA, USA), and then incubated the bag at 37°C for 30 minutes with gentle shaking to obtain ¹³CO-saturated autologous blood. The FCO_{Hb} of the blood was determined using a CO-hemoximeter integrated into a blood gas analyzer (ABL-720, Radiometer Copenhagen Co., Ltd., Copenhagen, Denmark), and the ¹³CO-saturated blood was infused back into the subject within 60 minutes.

During the infusion, venous blood was sampled every 5 minutes to determine the FCO_{Hb} using the ABL-720. The infusion was terminated when the FCO_{Hb} reached 9%.

Analysis of CO and ¹³CO₂ production

Following infusion, exhaled gas was collected in a 1.3-L bag, and the MVCO was measured every hour for the first 12 hours and every 4 hours thereafter, as was also done for baseline exhaled gas samples and MVCO measurement. Production of ¹³CO₂ was monitored by measuring increases in the ¹³CO₂/¹²CO₂ ratio in exhaled gas samples using an infrared spectral analyzer (POCone, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The POCone analyzer is capable of determining changes in the ¹³CO₂/¹²CO₂ ratio relative to baseline ($\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$) at a resolution of a part per thousand. A single measurement required 120 mL of sample gas, and the measurement was repeated 10 times for each sample.

Venous blood was sampled every 4 hours to measure FCO_{Hb}, and the ETCO was estimated simultaneously using the device described in **Figure 1**. The experiment was terminated 36 hours after infusion, when the $\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$, FCO_{Hb}, MVCO, and ETCO values had returned to baseline levels.

Quantitative analysis

The infused volume of CO (¹³CO) was calculated from the FCO_{Hb}, solubility of CO, and volume of CO-saturated blood infused. We also calculated the total volumes of CO and CO₂ exhaled in the 37-hour period from the beginning of the infusion step until the end of the experiment by adding the measured MVCO and MVCO₂ values. The volume of endogenous CO exhaled during the experimental period was estimated from the baseline MVCO and then subtracted from the total to obtain the volume of exogenous CO derived from the infusion that was exhaled during the experimental period. From these values, the volume and percentage of the infused CO that was then either exhaled or retained in the body were estimated. The volume and percentage of

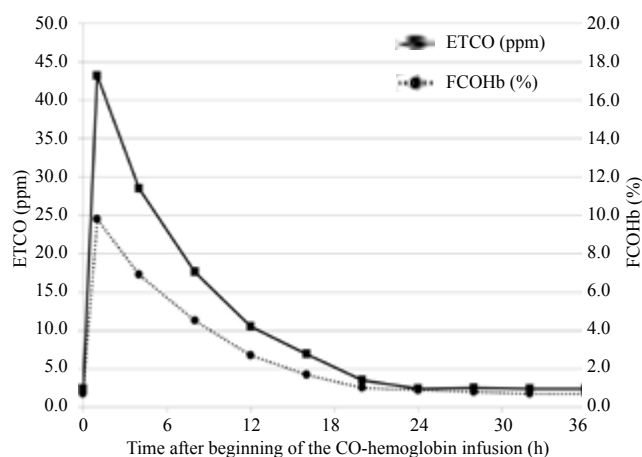


Figure 2: Changes in ETICO and FCOHb after beginning of CO-hemoglobin infusion.

Note: Closed boxes with solid line show the change in end-tidal breath CO (ETICO, left axis) concentration over time. Closed circles with dotted line show the change in carboxyhemoglobin fraction in the venous blood (FCOHb, right axis) over time. CO: Carbon monoxide; h: hour(s).

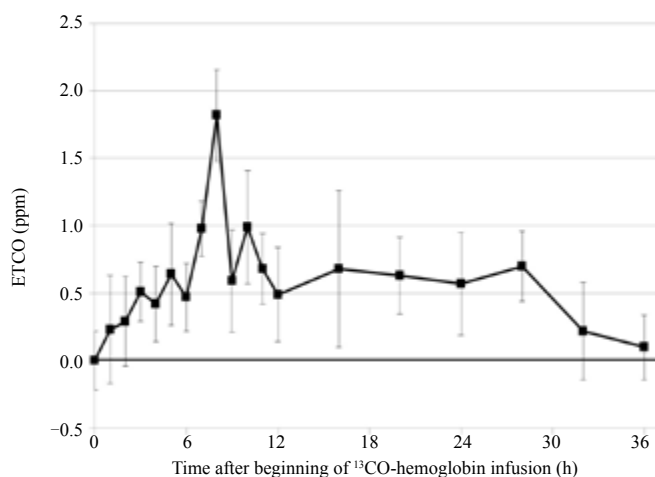


Figure 3: Change in the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio relative to baseline ($\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$; per mil) over the course of the experiment.

Note: Closed boxes and error bars represent means and 95% confidence intervals of 10 repeated measurements per sample. CO: Carbon monoxide; CO_2 : carbon dioxide; h: hour(s).

the infused ^{13}CO that was oxidized and exhaled as $^{13}\text{CO}_2$ were estimated from the MVCO_2 and $\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$ time-course data.

Statistical method

We applied Student's *t*-test and 5% significance level to assess the significance of increase in $^{13}\text{CO}_2$ production.

RESULTS

Time-course changes of end tidal CO and CO-hemoglobin fraction

Figure 2 shows the changes in ETICO and FCOHb during

Table 1: Summary of the fate of CO in the body, beginning at the time of CO infusion

Fate of CO	Volume (L)
Volume of infused CO (*1)	1.5×10^{-1}
Hemoglobin bound	1.3×10^{-1}
Solved	1.8×10^{-2}
Volume of exhaled CO (*2)	1.5×10^{-1}
Endogenous (baseline)	3.0×10^{-2}
Exogenous (infused)	1.2×10^{-1}
Destinations of infused CO (*3)	
Exhaled	1.2×10^{-1} (81%)
Not exhaled (retained in the body)	2.8×10^{-2} (19%)
Volume of exhaled $^{13}\text{CO}_2$ (from infused ^{13}CO) (*4)	5.2×10^{-3}
Exhaled $^{13}\text{CO}_2$ /Infused ^{13}CO (*5)	4.9%
Exhaled $^{13}\text{CO}_2$ /Infused & retained ^{13}CO (*6)	26%

Note: *1: The sum of the CO bound to hemoglobin and dissolved in the plasma.

*2: The volume of CO exhaled, which was derived from either endogenous production or infusion (exogenous). *3: The volume and the percentage of infused CO (^{13}CO) that was either exhaled over the course of the experiment or retained in the body. *4: The volume of $^{13}\text{CO}_2$ derived from oxidation of the infused ^{13}CO that was then exhaled during the experiment. *5: The percentage of $^{13}\text{CO}_2$ exhaled relative to the infused volume of ^{13}CO . *6: The percentage of $^{13}\text{CO}_2$ exhaled relative the estimated volume of ^{13}CO not exhaled and retained in the body. CO: Carbon monoxide; CO_2 : carbon dioxide.

the course of the experiment. Following infusion, the ETICO and FCOHb increased to 43.2 ppm and 9.8%, respectively. Thereafter, both parameters exhibited almost identical rates of exponential decay and returned to baseline levels after 24 hours.

Time-course change of exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio relative to the baseline

Values for $\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$ over the course of the experiment are shown in Figure 3. The $\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$ increased significantly ($P < 0.05$, vs. 0 hour) from 3 hours to 28 hours after the initiation of ^{13}CO -hemoglobin infusion, with a peak at 8 hours.

Time-course changes of MVCO and minute volume of $^{13}\text{CO}_2$ ($\text{MV}^{13}\text{CO}_2$) exhalations

Changes in the MVCO and $\text{MV}^{13}\text{CO}_2$ exhalations from the initiation of infusion until the end of the experiment are shown in Figure 4.

The fate of CO in the body

The quantitative summary of the production and the fate of CO in the body, beginning at the time of CO infusion, until the end of the experiment, is shown in Table 1.

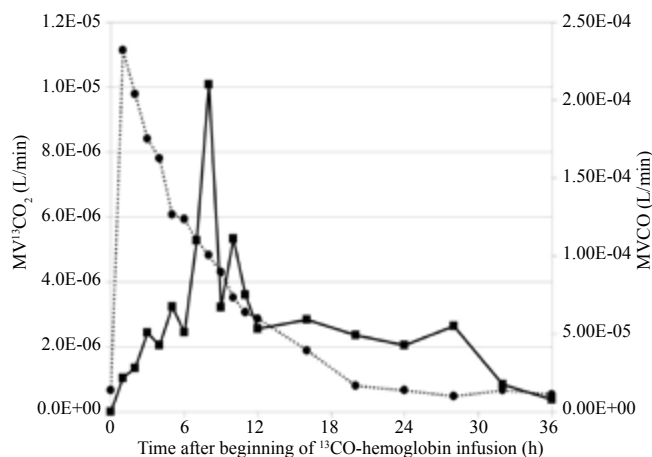


Figure 4: Changes in the MVCO and MV¹³CO₂ exhalation from the initiation of infusion.

Note: Closed boxes and solid line show the change in the minute volume of ¹³CO₂ (MV¹³CO₂) exhalation derived from ¹³CO-hemoglobin infusion (left axis) over time. Closed circles and dotted line show the change in minute volume of CO exhalation (MVCO, right axis) over time. CO: Carbon monoxide; CO₂: carbon dioxide; h: hour(s).

DISCUSSION

Previous studies have described the residual effects of CO after carboxyhemoglobin elimination in CO-intoxicated patients (Halperin et al., 1959), the toxic effects of chronic exposure to low-concentration CO (Wang, 2004), the poor correlation between blood carboxyhemoglobin levels and the physiologic effects of CO inhalation (Stewart, 1975), and the protective effect against ischemia-reperfusion injury associated with CO inhalation in rats (Fujimoto et al., 2004). These physiologic effects associated with CO inhalation cannot be explained by carboxyhemoglobin-induced hypoxia alone and instead suggest that CO is redistributed from the blood hemoglobin into tissue cells, where it activates or inhibits various heme protein enzymes (Coburn and Mayers, 1971; Piantadosi, 2002). However, such a redistribution of CO under physiologic conditions has yet to be demonstrated (Wu and Wang, 2005).

A previous study reported the production of ¹³CO₂ in a human volunteer following the inhalation of 50 ppm of ¹³CO gas (Sawano and Shimouchi, 2010). In that study, human blood was circulated through a cardiopulmonary bypass circuit that simulates human blood circulation and gas exchange, with 50 ppm of ¹³CO gas supplied to the oxygenator. However, no ¹³CO₂ production was detected. These results demonstrated that under physiologic conditions CO is oxidized within the tissues rather than in the circulating blood (Sawano and Shimouchi, 2010). Due to the possibility that the ¹³CO₂ detected could have been derived from the oxidation of CO in the airway epithelium, however, the authors of that study were unable to definitively demonstrate the redistribution of CO from the blood to the tissues.

Another study reported a significant increase in $\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$ between 4 and 31 hours, with a peak at 9 hours after ¹³CO inhalation, which exposed the airway epithelium to 50 ppm of ¹³CO for 4 hours (Sawano and Shimouchi, 2010). Thus, if the oxidation of CO occurs primarily in the airway epithelium, the increase and peak in ¹³CO₂ production following ¹³CO inhalation should have appeared 4 hours earlier compared with ¹³CO-hemoglobin infusion. However, the time course of ¹³CO₂ production following inhalation and infusion were almost identical in this study and the past, Sawano and Shimouchi (2010) suggesting that CO oxidation does not occur in the airway epithelium.

In the present study, quantitative analyses revealed that approximately 20% of the infused hemoglobin-bound CO was not exhaled between the initiation of infusion and termination of the experiment and was instead retained in the body. The ETCO, blood FCOHb, MVCO, MV¹³CO₂ and $\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$ all returned to baseline levels at the end of the experimental period (Figures 2–4). Therefore, we conclude that the percentage of the infused CO that was retained in the body had been redistributed from the blood into the tissue cells. Taken together, these results demonstrate that a portion of hemoglobin-bound CO moves from the blood to the tissues, where it is then oxidized. The elucidation of this pathway for the redistribution of CO from the circulating blood to the tissues strongly suggests that the role of endogenous CO is not limited to intracellular signal transmission but may extend to systemic or inter-organ signal transmission.

Previous studies estimated that 80% of the total CO body store is bound to hemoglobin in the red blood cells as carboxyhemoglobin and that 20% is bound to intracellular heme proteins (Coburn, 1967, 1970). Another study suggested that these CO body stores are exchangeable and that CO moves from the blood to the tissues, where it binds to heme proteins (Coburn and Mayers, 1971). It is interesting that the ratio of the redistribution of CO from the blood to the tissues revealed by our quantitative analysis in the present study corresponds well with determinations of the distribution of CO body stores identified in these studies. However, the present study included only one subject; thus, further investigations involving more subjects are needed to derive definite conclusions. Another limitation of our quantitative analysis is that we did not measure CO emission from the skin. However, another study reported that the MVCO emission from the skin is no greater than 0.25% of the exhalation level (Nose and Shimouchi, 2008), suggesting that this omission did not significantly affect the results of the present study.

In conclusion, our study demonstrated that a portion of hemoglobin-bound CO is redistributed from the blood to



the tissues, where it is then oxidized. However, the enzyme responsible for the oxidation of CO in the tissues of the human body remains to be identified. Several laboratory studies have demonstrated this enzymatic reaction using cytochrome C oxidase extracted from mitochondria of various animal organs (Tzagoloff and Wharton, 1965; Young and Caughey, 1986; Vijayarathy et al., 1999), but no study has traced the reaction in live human subjects. The enzyme is localized in mitochondria, which are eliminated from red blood cells during the course of their maturation (Zhang et al., 2009). Our study demonstrated that the oxidation of CO does not occur in the circulating blood and suggests that the enzyme responsible for the reaction is located in the tissues. However, further investigation is needed to test this hypothesis.

A part of this paper was presented at the Breath Summit 2015, the scientific meeting of the International Association for Breath Research, 13–15 Sept. 2016, Vienna, Austria.

Author contributions

MS exclusively contributed to the study, read and approved the final version of this paper.

Conflicts of interest

None declared.

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