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

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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 13CO₂ production following 13CO gas inhalation demonstrated that CO is oxidized to carbon dioxide (CO₂) 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 13CO₂ production following 13CO-hemoglobin infusion. The subject was infused with 0.45 L of 13CO-saturated autologous blood. Exhaled gas was collected intermittently for 36 hours for measurement of minute volumes of CO/CO₂ exhalation and determination of the 13CO₂/12CO₂ ratio. 13CO₂ 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 13CO₂. Identical time courses of 13CO₂ production following 13CO-hemoglobin infusion and 13CO 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 13CO₂ production following the inhalation of 13CO 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 13CO₂ production following infusion of 13CO-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.
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 (FCOHb 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 FCOHb 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 FCOHb using the ABL-720. The infusion was terminated when the FCOHb 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 (Δ13CO2/12CO2) 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 FCOHb, and the ETCO was estimated simultaneously using the device described in Figure 1. The experiment was terminated 36 hours after infusion, when the Δ13CO2/12CO2, FCOHb, MVCO, and ETCO values had returned to baseline levels.

Quantitative analysis
The infused volume of CO (13CO) was calculated from the FCOHb, 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
the course of the experiment. Following infusion, the ETCO 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.

The time-course change of exhaled \(^{13}\)CO\textsubscript{2}/\(^{12}\)CO\textsubscript{2} ratio relative to the baseline

Values for \(\Delta^{13}\)CO\textsubscript{2}/\(^{12}\)CO\textsubscript{2} over the course of the experiment are shown in Figure 3. The \(\Delta^{13}\)CO\textsubscript{2}/\(^{12}\)CO\textsubscript{2} increased significantly \((P < 0.05, \text{vs. } 0 \text{ hour})\) from 3 hours to 28 hours after the initiation of \(^{13}\)CO-hemoglobin infusion, with a peak at 8 hours.

The time-course changes of MVCO and minute volume of \(^{13}\)CO\textsubscript{2} (MV\(^{13}\)CO\textsubscript{2}) exhalations

Changes in the MVCO and MV\(^{13}\)CO\textsubscript{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.

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\textsuperscript{-1} |
| Hemoglobin bound                       | 1.3×10\textsuperscript{-1} |
| Solved                                 | 1.8×10\textsuperscript{-2} |
| Volume of exhaled CO (*2)              | 1.5×10\textsuperscript{-1} |
| Endogenous (baseline)                  | 3.0×10\textsuperscript{-2} |
| Exogenous (infused)                    | 1.2×10\textsuperscript{-1} |
| Destinations of infused CO (*3)        |            |
| Exhaled                                | 1.2×10\textsuperscript{-1} |
| Not exhaled (retained in the body)     | 2.8×10\textsuperscript{-3} |
| Volume of exhaled \(^{13}\)CO \textsubscript{2} (from infused \(^{13}\)CO) (*4)| 5.2×10\textsuperscript{-3} |
| Exhaled \(^{13}\)CO\textsubscript{2}/Infused \(^{13}\)CO (*5) | 4.9% |
| Exhaled \(^{13}\)CO\textsubscript{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 \(^{13}\)CO\textsubscript{2} derived from oxidation of the infused \(^{13}\)CO that was then exhaled during the experiment. *5: The percentage of \(^{13}\)CO exhaled relative to the infused volume of \(^{13}\)CO. *6: The percentage of \(^{13}\)CO exhaled relative the estimated volume of \(^{13}\)CO not exhaled and retained in the body. CO: Carbon monoxide; CO\textsubscript{2}: carbon dioxide.
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 $^{13}$CO$_2$ in a human volunteer following the inhalation of 50 ppm of $^{13}$CO 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 $^{13}$CO gas supplied to the oxygenator. However, no $^{13}$CO$_2$ 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 $^{13}$CO$_2$ 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 $\Delta^{13}$CO$_2$/$^{12}$CO$_2$ between 4 and 31 hours, with a peak at 9 hours after $^{13}$CO inhalation, which exposed the airway epithelium to 50 ppm of $^{13}$CO for 4 hours (Sawano and Shimouchi, 2010). Thus, if the oxidation of CO occurs primarily in the airway epithelium, the increase and peak in $^{13}$CO$_2$ production following $^{13}$CO inhalation should have appeared 4 hours earlier compared with $^{13}$CO-hemoglobin infusion. However, the time course of $^{13}$CO$_2$ 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 FC0Hb, MVCO, MV$^{13}$CO$_2$, and $\Delta^{13}$CO$_2$/$^{12}$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; Vijayasarathy 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.

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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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