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A Tracer Analysis Study on the Redistribution and Oxidization of Endogenous Carbon Monoxide in the Human Body

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Summary **Past studies have suggested that some carbon monoxide (CO) moves from blood haemoglobin to tissue cells and that mitochondrial cytochrome c oxidase oxidizes CO to carbon dioxide (CO_2). However, no study has demonstrated this redistribution and oxidization of CO under physiological conditions. The objective of this study was to trace the redistribution and oxidization of CO in the human body by detecting $^{13}CO_2$ production after the inhalation of ^{13}CO. In Experiment 1, we asked a healthy subject to inhale 50 ppm ^{13}CO gas. In Experiment 2, we circulated heparinized human blood in a cardio-pulmonary bypass circuit and supplied 50 ppm ^{13}CO gas to the oxygenator. We sequentially sampled exhaled and output gases and measured the $^{13}CO_2/^{12}CO_2$ ratios. In Experiment 1, the exhaled $^{13}CO_2/^{12}CO_2$ ratio increased significantly between 4 to 31 h of ^{13}CO inhalation. In Experiment 2, the output $^{13}CO_2/^{12}CO_2$ ratio showed no significant increase within 36 h of ^{13}CO input. Experiment 1 demonstrated the oxidization of CO in the human body under physiological conditions. Experiment 2 confirmed that oxidization does not occur in the circulating blood and indicated the redistribution of CO from blood carboxyhaemoglobin to tissue cells.**

Key Words**: carbon monoxide, redistribution, oxidization, tracer analysis, stable isotope**

Introduction

Since endogenous carbon monoxide (CO) production by heme degradation was first reported in the early 1950s [*1*, *2*], numerous studies have revealed its physiological roles and regulatory mechanisms [*3*]. However, two pathways in the disposition and catabolism of endogenous CO remain unidentified. One pathway is the redistribution of CO from circulating blood to tissue cells. It is supposed that 80% of endogenously produced CO binds to haemoglobin in the circulating blood and is finally exhaled [*4*]. Although several studies have suggested that a portion of CO moves from blood haemoglobin to tissue and binds to intracellular heme proteins [*5–8*], this redistribution of CO has not been demonstrated under physiological conditions [*3*]. Another pathway is the oxidization of CO to carbon dioxide (CO_2) by cytochrome c oxidase in mitochondria. It has been believed that a small metabolic endpoint of endogenous CO is the oxidization to CO_2 [*9*] a process that has been demonstrated *in vitro* [*10–12*]. However, *in vivo* oxidization of CO under physiological conditions has not been shown [*3*].

The objective of this study was to trace these unidentified redistribution and oxidization pathways of CO in the human body through detection of $^{13}CO_2$ production by exhaled gas analysis following the inhalation of ^{13}CO gas. ^{13}C is a stable isotope of ^{12}C with an abundance ratio of 1.1%.

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Experimental Procedures

The experimental protocol of this study was approved by the ethics committee of Saitama Medical University.

Experiment 1

The subject was a healthy male adult volunteer with no smoking habits who gave written informed consent. First, the subject inhaled the synthesized air and exhaled into a 1.3-L gas sampling bag (Pylori exhaled gas sampling bag, Fukuda Denshi Co., Ltd., Tokyo, Japan) after holding his breath for 20 s. Twenty-five bags of exhaled gas were collected as the background.

Then the subject inhaled synthesized air that contained 50 ppm ^{13}CO gas. During this procedure, a physician closely observed the subject and monitored his electrocardiogram, pulse-oximetry, and blood pressure in an operating room equipped with a forced ventilation system. Exhaled CO and CO_2 concentrations were continuously analyzed by an electrochemical sensor (Carbolizer mBA-1000, Taiyo Co., Ltd., Osaka, Japan). This sensor is capable of determining CO and CO_2 concentrations every second with resolutions of 0.1 ppm and 0.1%, respectively [*13*]. The procedure was terminated before the exhaled CO concentration achieved 50 ppm, which is almost equivalent to 10% blood carboxyhaemoglobin concentration (COHb%). Following the inhalation of ^{13}CO gas, exhaled gas was sampled every hour for the first 12 h, every 2 h for the next 12 h, and thereafter every 4 h, by the same procedure as the background. We also sampled venous blood every 6 h to measure COHb% by a CO-hemoximtre integrated into a blood gas analyzer (ABL-720, Radiometer Copenhagen Co., Ltd., Copenhagen, Denmark). In this study, we detected $^{13}CO_2$ production by measuring the increase of the $^{13}CO_2/^{12}CO_2$ ratio in exhaled gas using an infrared spectral analyzer (POCone, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). This analyzer is capable of determining changes in the $^{13}CO_2/^{12}CO_2$ ratio with a resolution of 0.1 per mil (0/00) relative to the background. A single measurement required 120 mL of sample and the background gases, and the measurement was repeated ten times for each sample. We terminated the experiment 36 h after inhalation, when the elevated $^{13}CO_2/^{12}CO_2$ ratio of the sample had recovered to the background level.

Experiment 2

The objective of Experiment 2 was to ensure that the $^{13}CO_2$ production shown in Experiment 1 was totally derived from the oxidization of ^{13}CO in tissue cells. Fig. 1 shows the experimental device that simulates human blood circulation and gas exchange on a 1/10 scale. We filled a CAPIOX cardiopulmonary bypass circuit (Terumo Co., Tokyo, Japan) with 400 mL of human whole blood, 120 ml of saline, and 1000 units of heparin. A centrifugal pump regulated the circulating blood flow at 0.5 l/min. We infused 100% CO_2 gas into the circuit to simulate tissue CO_2 production.

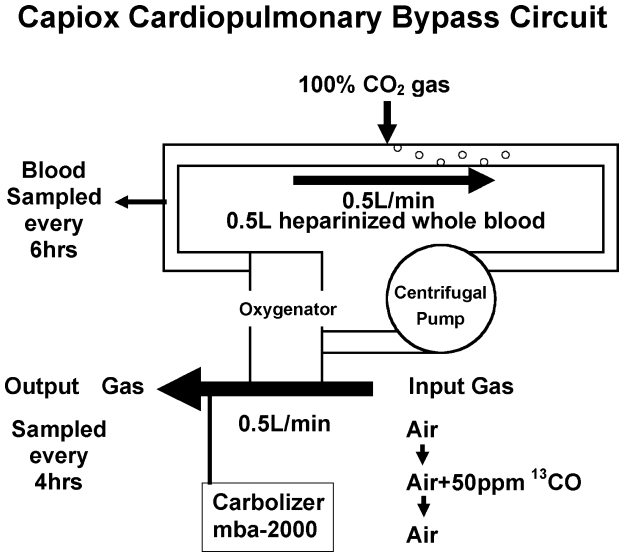

Fig. 1. Schematic of the device used for Experiment 2. The centrifugal pump circulates heparinized blood in a cardiopulmonary bypass circuit. Carbon dioxide gas is infused into the circuit. ^{13}CO is added to the input gas of the oxygenator and the output gas is sampled sequentially.

First, we supplied synthesized air at 0.5 l/min to the oxygenator as input gas, adjusted the infusion rate of CO_2 to maintain the output CO_2 concentration between 5% and 6%, and collected the output gas in the sampling bag as the background. Next, we added 50 ppm ^{13}CO to the input gas until the carboxyhaemoglobin concentration of the blood exceeded 10%. Then we collected output gas every 4 h for 36 h.

We measured the $^{13}CO_2/^{12}CO_2$ ratios of the output gas and the carboxyhaemoglobin concentration of the blood by the same procedures as in Experiment 1.

Statistical processing

The significance of the increase of the $^{13}CO_2/^{12}CO_2$ ratios was statistically assessed using Student's *t* test, with significance level of 5% ($p<0.05$).

Results

Figs. 2 and 3 show the increase in the exhaled $^{13}CO_2/^{12}CO_2$ ratios relative to the background and the blood carboxyhaemoglobin concentrations plotted against time in Experiments 1 and 2. Error bars of the $^{13}CO_2/^{12}CO_2$ ratios indicate two standard deviations or 95% confidence intervals of ten repeated measurements.

In Experiment 1, the exhaled $^{13}CO_2/^{12}CO_2$ ratio increased significantly between 4 and 31 h after ^{13}CO inhalation

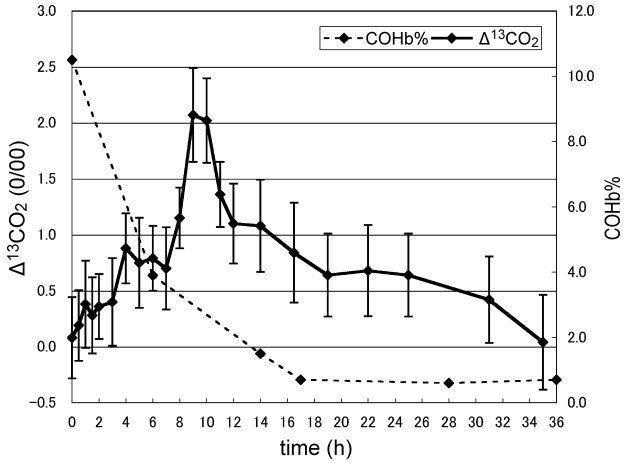

Fig. 2. Time course of $^{13}CO_2/^{12}CO_2$ ratio and blood carboxyhaemoglobin concentration for Experiment 1. Solid line represents the sequential increase in the $^{13}CO_2/^{12}CO_2$ ratios relative to the background (per mil, left axis). Broken line represent the sequential measurements of the blood carboxyhaemoglobin concentration (percentage, right axis). The error bars indicate two standard deviations or 95% confidence intervals of ten repeated measurements.

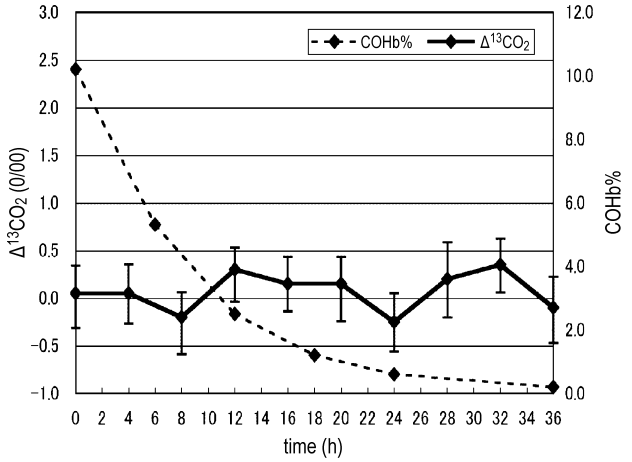

Fig. 3. Time course of $^{13}CO_2/^{12}CO_2$ ratio and blood carboxyhaemoglobin concentration for Experiment 2. Solid line represents the sequential increase in the $^{13}CO_2/^{12}CO_2$ ratios relative to the background (per mil, left axis). Broken line represents the sequential measurements of the blood carboxyhaemoglobin concentration (percentage, right axis). The error bars indicate two standard deviations or 95% confidence intervals of ten repeated measurements.

($p<0.05$). The maximum increase in the $^{13}CO_2/^{12}CO_2$ ratio was $+2.1_{0/00}$ of the background 9 h after inhalation. In Experiment 2, the output $^{13}CO_2/^{12}CO_2$ ratio showed no significant increase for 36 h after the ^{13}CO input.

Discussion

First, we ensured the subject was not subjected to symptomatic CO intoxication in Experiment 1. The threshold level for the carboxyhaemoglobin concentration to manifest the mildest symptoms of CO intoxication has reported to be 10% [*14*, *15*]. We have developed a system for non-invasive, continuous carboxyhaemoglobin densitometry by expiratory gas analysis that enables continuous real-time monitoring of the carboxyhaemoglobin concentration using the Carbolizer sensor [*13*]. Using this technique, we controlled the duration of ^{13}CO gas inhalation to limit the maximum carboxyhaemoglobin concentration of the subject to below 10%. In addition to the monitors equipped in the intensive care unit of our hospital, at least two physicians continuously monitored the subject's consciousness and neurological status for any unexpected manifestations during the experiment.

Over the past decade, numerous studies have clarified the physiological role of endogenous CO as a gas transmitter that activates or inhibits various enzymes by binding to heme proteins within a cell or adjacent cells. However, most researchers are sceptical about the role of CO as a transorganic or systemic signal transmitter, because the redistribution of CO from blood carboxyhaemoglobin to tissue cells has not been shown. It is widely accepted that the only destination for haemoglobin-bound CO is to be exchanged with oxygen in the lung and to be exhaled. Previous studies have shown remnant effects of CO after carboxyhaemoglobin elimination [*5*], a poor correlation of blood carboxyhaemoglobin levels with the physiological effects of CO inhalation [*7*], and a protective effect of CO inhalation against ischemia-reperfusion injury in rats [*8*]. These findings suggest that a fraction of CO moves from blood to tissue, but no tracer analysis study has demonstrated this redistribution.

Meanwhile, several laboratories have confirmed the oxidization of CO by mitochondrial cytochrome c oxidase extracted from various animal organs [*10–12*]. However, those studies detected CO_2 production from the purified enzyme gassed with CO in a closed chamber and no tracer analysis study has demonstrated the oxidization of CO in living animal tissue under physiological conditions.

In Experiment 1, we detected $^{13}CO_2$ production deriving from inhaled ^{13}CO, which demonstrated the oxidization of CO in a living human body under physiological conditions. In Experiment 2, we detected no $^{13}CO_2$ production, which confirmed that oxidization does not occur in the circulating blood. Taken together, these experiments indicate that a portion of inhaled CO is redistributed from the blood to tissue cells and is oxidized there.

There is still the possibility that CO oxidization occurs in airway epithelium. Inhaled CO contacts airway epithelium before it binds to blood haemoglobin and we may have

detected $^{13}CO_2$ that is derived from the oxidation of CO in the airway epithelium tissue. If the oxidization occurs in the airway, $^{13}CO_2$ production would increase during ^{13}CO inhalation and decrease soon after the termination of inhalation. However, a significant increase in $^{13}CO_2$ production started 4 h after the termination of ^{13}CO inhalation and was sustained for 31 h, with a peak at 9 h in Experiment 1. This time delay between CO inhalation and CO_2 production may reflect the time required for redistribution and thus refuting the hypothesis of CO oxidization in the airway epithelium. Still, further evidence is required to confirm such CO redistribution, including the detection of $^{13}CO_2$ production after infusion of ^{13}CO-bound carboxyhaemoglobin (^{13}CO-saturated autologous blood).

Acknowledgments

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