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In Vitro Absorption of Atmospheric Carbon Monoxide and Hydrogen Cyanide in Undisturbed Pooled Blood

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16. Abstract

Biological samples from victims of aircraft accidents are analyzed for carboxyhemoglobin (COHb) and cyanide ion (CN^-) in blood. Such victims quite often suffer large open wounds near the autopsy blood collection sites. Many aircraft crashes result in fires that fill the victim's atmosphere with smoke rich in carbon monoxide (CO) and hydrogen cyanide (HCN). It is important to determine whether pooled blood in those open wounds may have absorbed CO and HCN after death, which could lead one to erroneously conclude that the presence of COHb and CN^- in blood was the result of breathing in these combustion gases. A chamber was designed from a laboratory desiccator to establish whether CO and HCN may be absorbed in undisturbed, pooled whole human blood. A magnetic stirring bar was placed at the bottom of the chamber to facilitate air movement. A ceramic plate with concentric rows of holes was above the stirring bar to support a shallow open dish containing 4 mL of heparinized blood. Gas syringes (100-cc) were used to evacuate air from and add pure CO into the chamber. The chamber volume was 9038 cc. The blood was exposed to three concentrations of CO each for two different periods of time. For HCN exposures, an extra dish containing a 5-mL beaker, which contained sodium cyanide (NaCN), was used. Four mL of heparin-treated blood was used in the second dish. One mL of concentrated sulfuric acid was added to the beaker containing NaCN through the lid opening. The volume of the HCN chamber was 8981 cc. Blood COHb and CN^- concentrations were determined spectrophotometrically. COHb levels of 4.3-11.0% were detected in blood after its exposure to CO at 5532, 8298, 11064, 22129, and 33193 ppm for 30- and 60-min. CN^- concentrations (1.43-5.01µg/mL) in blood increased with exposure to HCN at 100 and 200 ppm each at 15, 30, 45, and 60 min. Increases in the COHb levels observed in these experiments do not exclude the possibility for higher levels of COHb in blood exposed to highly CO-rich atmospheres from actual fires. It was clearly evident that there is a strong potential for CN^- levels to increase by the absorption of atmospheric HCN. This selective absorption is consistent with the insolubility of CO and solubility of HCN in water. Thus, postmortem COHb and CN^- levels should be carefully interpreted.

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INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI; Oklahoma City, OK) assists in the investigation of fatal aircraft accidents by conducting toxicological analyses of biological specimens collected from the victims of the accidents (1,7). These biological samples are submitted by the local pathologists to CAMI in coordination with the FAA's Office of Accident Investigation and Prevention and authorized by the National Transportation Safety Board (NTSB). The NTSB investigates aviation accidents that occur within the jurisdiction of the United States.

One aspect of the toxicological analyses is the determination for the presence of primary combustion gases in blood specimens. Combined with the crash site investigation, autopsy and pathology findings, and toxicology results, the investigators could determine whether the crewmembers were incapacitated by engine carbon monoxide (CO) leaks into the cabin area, whether they survived the crash and were overcome by inhaling CO and hydrogen cyanide (HCN) from aircraft fires, whether the victims died on impact, or they came to a rapid death from the intense heat of the fire, without inhaling these gases.

Because of the violent impacts involved in crashes, victims quite often suffer large open wounds near sites on the body from where autopsy whole blood is collected. Many aircraft crashes result in fire, which, in turn, fill the atmosphere around the victims with CO and HCN laden smoke. Therefore, it is important to determine whether pooled blood in those open wounds may have absorbed CO and HCN present in the atmosphere after death and could lead investigators to erroneously conclude that the presence of carboxyhemoglobin (COHb) and cyanide ion (CN^-) in whole blood was the result of breathing in primary combustion gases. For this, an *in vitro* study was conducted to evaluate the absorption of these fire gases in undisturbed pooled human blood.

MATERIALS AND METHODS

Materials

All reagents, chemicals, and solvents were of analytical grade and were of the highest available purity. Aforementioned items, standards, and other laboratory supplies were obtained from commercial sources. CO of > 99% purity was obtained in a compressed gas cylinder from Airgas (Oklahoma City, OK).

Human Blood

Blood samples were collected from healthy volunteers. The human subject participation was approved by the CAMI Institutional Review Board. The subjects were not on any medication and were non-smokers. Blood was collected in 10-ml green-top glass tubes at the CAMI Clinic. These tubes were sterile and contained sodium heparin. The collected blood was stored at 0–4°C. Prior to the absorption experiments, the collected blood was brought to the room temperature and was mixed by slowly inverting the blood tubes several times.

Exposure Chamber

A large glass vacuum laboratory desiccator with a ceramic plate (platform) with concentric rows of holes was used as an exposure chamber. The plate was located in the center to horizontally partition the desiccator and served as a platform for placing a blood sample in an open glass stender dish (37 mm x 25 mm; Carolina Biological Supply Company, Burlington, NC). Other items, including any liquid in a container, needed for the experiment were also placed on the platform. The tapered sleeve valve of the desiccator lid was configured with a female Luer-Lok fitting affixed with a small section of Tygon® tubing. Air was evacuated and CO was added to the chamber by using 100-cc valved gas-tight syringes with male Luer-Lok fittings (Precision Sampling Corporation, Baton Rouge, LA) through the tapered sleeve valve. A minimal amount of silicone-based vacuum grease was used on the tapered sleeve valve, on the rims of the lid, and on the body of the desiccator. To mix and circulate the atmospheric gases within the chamber, a large cross-shaped Teflon-coated magnetic stirring bar was placed inside the chamber at the bottom. The desiccator assembly was placed on a magnetic stirring plate. The rotation of the magnetic bar was controlled by adjusting the knob of the stirring plate. Each experiment was performed with a 4-mL aliquot of blood in an open glass stender dish. All experiments were performed in triplicate at laboratory temperature in a fume hood (Hamilton Industries, Two Rivers, WI). After having been exposed to CO or HCN, the blood samples were refrigerated (0–4°C). They were then analyzed for COHb or CN^- in accordance with standard CAMI laboratory analytical procedures.

CO Absorption

Total volume of the chamber with the ceramic plate, stirring bar, and dish, including the water equivalent of 4 mL blood sample, was determined by measuring the volume of water necessary to fill the desiccator and its lid. The chamber volume was found to be 9.038 L. A CO gas cylinder with a pressure regulator was configured with a valve having a female Luer-Lok fitting affixed with a small piece of Tygon® tubing. After placing the magnetic stirring bar in the bottom and 4 mL human blood sample in an open stender dish on the ceramic plate in the desiccator, it was sealed with the lid. Measured volumes of air were removed from the chamber through the tapered sleeve valve and were replaced with the equivalent volumes of CO to reach the desired atmospheric concentrations of CO in the chamber. The removal of air and its replacement with CO was achieved by using the gastight syringe. After adding CO, the tapered sleeve valve was closed. Various volumes of CO corresponding to its atmospheric concentrations in the chamber are given in Table I. The undisturbed human blood samples in the dishes were exposed to the circulated CO atmosphere for 30 and 60 min. After the exposure period for each experiment, the chamber was carefully opened by sliding the lid, and the blood sample was transferred in three 0.5-mL screw-capped polypropylene tubes (Fisher Scientific, Pittsburgh, PA) for COHb analysis. These blood aliquots were refrigerated until analyzed.

HCN Absorption

The exposure apparatus was further modified for conducting HCN absorption experiments by using an open glass dish containing a 5-mL glass beaker having a weighed amount of sodium cyanide (NaCN). The ideal gas law was used to calculate the amount of the limiting reactant (i.e., NaCN) needed to react with sulphuric acid to achieve the required concentrations of HCN in the chamber. By water displacement, the volume of the chamber for HCN experiments was determined to be 8.981 L, after taking into account the volumes of the blood sample, sulfuric acid, and the items used in the desiccator. To conduct the experiment, 4 mL of blood was used in a stender dish. With the lid of the chamber partially opened, 1 mL of concentrated sulfuric acid was added to the beaker containing NaCN; then, the chamber lid was immediately closed. The tapered sleeve valve on the lid was in the closed position. The amount of NaCN in relation to sulfuric acid corresponded to an equivalent molar proportion to produce the desired amount of HCN ($H_2SO_4 + 2NaCN \rightarrow 2HCN + Na_2SO_4$). Using the gas law, the calculated final exposure concentrations of HCN in the chamber at the normal temperature and pressure were 100 and 200 ppm (μL/L). The HCN exposure time to the undisturbed pooled blood samples ranged from 15 to 60 min. After the exposure time, the chamber was carefully opened by sliding the lid, and the blood was transferred in three 1.5-mL screw-capped polypropylene tubes (Fisher Scientific, Pittsburgh, PA) for CN^- analysis.

Blood COHb Determination

Blood COHb concentrations in the specimens were determined spectrophotometrically (4,5,23,25). Blood cells were hemolyzed with ammonium hydroxide, and the hemolysate was treated with sodium dithionite to reduce methemoglobin and oxyhemoglobin to deoxyhemoglobin (HHb). The blood solution obtained was scanned in the visible range from 450 nm to 750 nm. The absorbance of the blood solution was recorded at 540 nm, a wavelength of maximum absorbance for COHb, and at 579 nm, a wavelength at which the spectra of various species of HHb have the same absorbance (isosbestic point). Ratios of the absorbance values of the specimens at these two wavelengths were used to determine %COHb in the specimen by using a mathematical equation in relation to a positive COHb control prepared in human blood by using CO (4,5).

Blood CN^- Measurement

HCN was liberated from the blood sample by acidification and microdiffusion, trapped in a dilute alkaline solution, and converted to cyanogen chloride after reacting with chloramine-T. Subsequently, cyanogen

Table I. Various Volumes of CO Corresponding to the Final Atmospheric Concentrations of the Gas in the Exposure Chamber

Volumes of CO Inserted in Chamber (cc; mL)	Final Concentration of CO in Exposure Chamber (ppm; μL/L)
50	5532
75	8298
100	11064
200	22129
300	33193

chloride was allowed to react with pyridine to form *N*-cyanopyridinium chloride, followed by a reaction wherein *N*-cyanopyridinium chloride was cleaved to form an anil of glutaconic aldehyde. This aldehyde then coupled with barbituric acid to a red-pinkish, highly resonant product (3,10,21). Appearance of a red-pinkish product suggested the presence of CN^-. The level of CN^- was quantitatively determined by measuring the absorbance of the product at 580 nm. Along with blood samples, a CN^--free human blood sample and 2 µg/mL CN^- control in human blood were also processed and analyzed. The absorbance of each solution at 580 nm against the solution obtained by processing water, as described above, was measured as soon as possible, since the color product produced is not very stable.

Statistics

The mean and standard deviation (SD) of quantitative analytical values for each analyte were calculated by using Microsoft® Office Excel 2003 (Redmond, WA). Significance of differences between means of observations was checked by Student's *t*-test. The difference between two means was considered significant, with $p < 0.05$.

RESULTS AND DISCUSSION

Under the experimental conditions, an increase of only a small amount of COHb was detected in the undisturbed whole blood after its exposure to CO at 5532, 8298, 11064, 22129, and 33193 ppm for 30 and 60 min. The COHb concentration in the blood was found to be 2.8% at no added atmospheric CO concentration, whereas the COHb levels ranged from 4.3 to 11.0% with 5532–33193 ppm CO. Except for the 11.0% COHb observed with 33193 ppm CO for the 60-min exposure, all other COHb values were below the laboratory cutoff of 10% (Fig. 1). The 11.0% COHb value was not statistically significant ($p = 0.07$) with the closest corresponding 60-min COHb value of 8.6% at 22129 ppm CO. The highest level of 11.0% COHb observed under the experimental conditions was barely above the laboratory cutoff. COHb at 10% is considered asymptomatic in humans (13,15), but tightness in forehead, possible slight headache, and dilation of cutaneous blood vessels are linked with 10–20% COHb (13,15). Normally, healthy individuals may accumulate up to 10% COHb from inhaling air contaminated with CO (2,24)—for example, from smoking, breathing gasoline exhaust fume rich air, or operating (travelling in) vehicles with faulty exhaust systems.

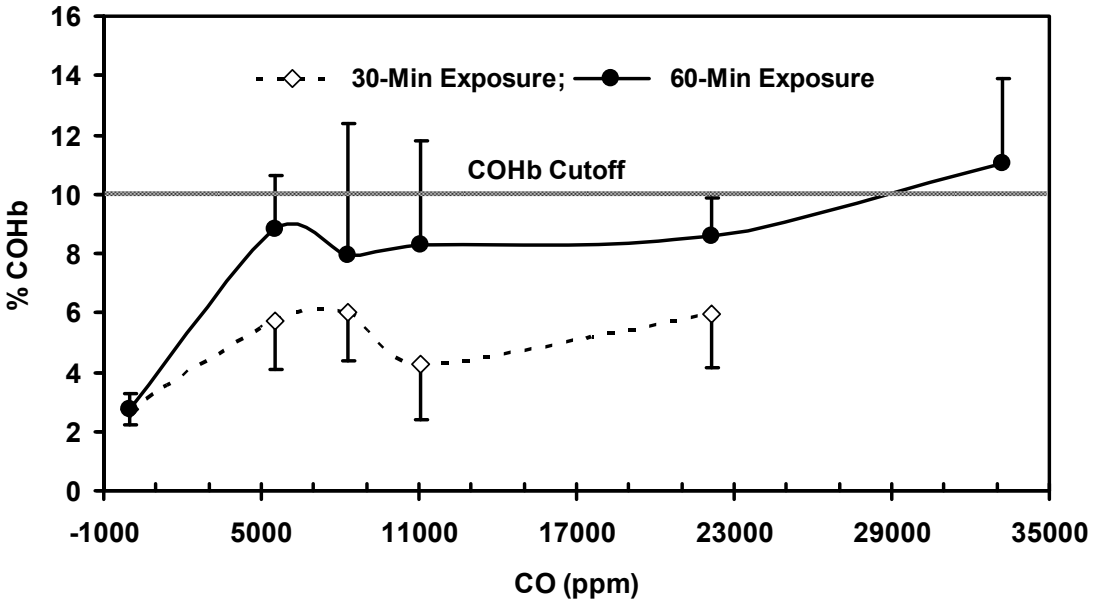

Figure 1. Passive absorption of CO at various atmospheric concentrations in undistributed pooled whole blood samples. The exposure times of the blood samples to CO were 30 and 60 min. The analytical cutoff value of the CAMI laboratory for reporting the COHb value is 10% and is shown in the figure by a horizontal line. Vertical lines shown in the curves are error bars. The details of the experimental design and analytical methods are given in the Materials and Methods section.

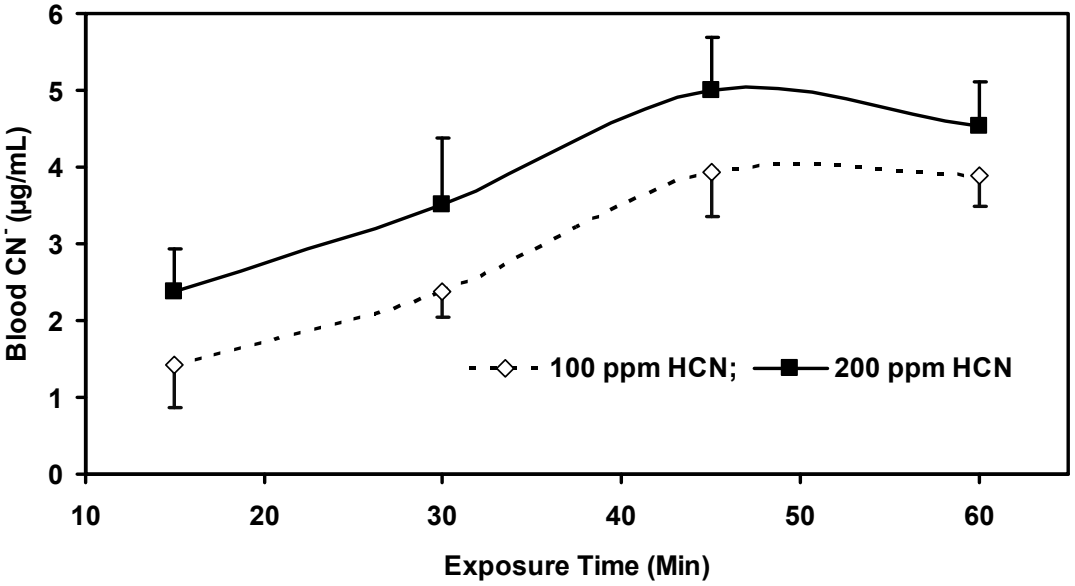

Figure 2. Passive uptake of HCN at 100 and 200 ppm atmospheric concentrations in undisturbed pooled whole blood samples. The exposure times of the blood samples to HCN at each atmospheric concentration were 15, 30, 45, and 60 min. Vertical lines shown in the curves are error bars. The analytical cutoff value of the CAMI laboratory for reporting the CN^- value is 0.25 µg/mL. The details of the experimental design and analytical methods are given in the Materials and Methods section.

Contrary to the COHb findings, CN^- concentrations in whole blood increased with exposure to an atmosphere containing HCN at 100 and 200 ppm each for 15, 30, 45, and 60 min. The CN^- concentration in blood ranged from 1.43 to 5.01 µg/mL. In humans, CN^- levels from 1.0 to ≥2.5 µg/mL could cause moderate-to-severe toxicity, producing mydriasis, cyanosis, and death (13,15). Under the experimental conditions, no CN^- was analytically detected in the blood sample not exposed to HCN. As depicted in Figure 2, the passive uptake of HCN increased with a function of HCN concentration and exposure time. The 15-min CN^- values of 100 and 200 ppm exposures were different from each other ($p < 0.05$). Similarly, the 30-, 45-, and 60-min values of 100 and of 200 ppm HCN were respectively different from the corresponding time point values. After the 45-min exposure, the CN^- concentration plateaued or even became less. The CN^- concentrations for 100 ppm HCN were significantly different ($p < 0.05$) with 15-min versus 30-min values and 30-min versus 45-min values, but the values at 45 min and 60 min were not significant ($p = 0.90$). With 200 ppm HCN experiments, the CN^- concentration also significantly increased up to 45 min ($p < 0.05$); although the CN^- values at 45 min and 60 min were significantly different from each other ($p < 0.05$), there was a decrease at 60 min instead of an increase. In relation to the 45-min CN^- concentration, the 60-min value was 9% less ($p = 0.034$). Such a decrease in the blood CN^- value might be related to a saturation of the HCN uptake system of the blood and a possible conversion of the absorbed HCN into some other chemical form(s) such as thiocyanate (13). That type of conversion may lead to the unaccountability for the total absorbed HCN, as all CN^- may not be available for the analytical reaction.

It is a known fact that each fire is different. Types and amounts of combustion products generated during fires can vary from fire to fire (6,11,20,22). Such variations depend upon the physical and chemical characteristics and amounts of the burning material and environmental conditions; for example, heat and air supply. In air transport cabin mockup fire experiments, consisting of passenger seats, urethane seat foam, and other materials under different burning conditions, CO has been recorded to be present in concentrations ranging from < 0.1% (1000 ppm) to > 1.5% (15000 ppm) (18). In this 1970 study, HCN (10 ppm) was reported to be present in only one fire experiment. CO concentrations up to 14% (140000 ppm) and HCN up to 1721 ppm have been reported in different combustion experiment atmospheres (8,9,12,14,16,17,19). The CO (5532–33193 ppm) and HCN (100 and 200 ppm) concentrations used in the present *in vitro* study fall within the concentration ranges of these gases reported in the experimental fire atmospheres.

Although findings of the present study demonstrated that the COHb level in the undisturbed pooled blood exposed to an atmosphere containing CO within the

parameters of this experiment would increase only up to 11%, it does not necessarily exclude the possibility that the COHb level in pooled blood from an actual fire victim may be higher than 11% if that blood were exposed to a very high concentration of atmospheric CO from an actual fire. Such COHb increase would occur because of the high concentration-gradient between blood and CO-rich air. Increase in the COHb level would also be possible if the pooled blood were disturbed, thereby producing a larger dynamic exposure surface area. With HCN, however, there is a strong clear-cut evidence that CN^- levels would easily increase in the undisturbed pooled blood due to the absorption of atmospheric HCN present in the fire environment.

This selective absorption pattern of the two primary combustion gases is consistent with the insolubility of CO and solubility of HCN in water, thus in the whole blood. The present study suggests that the blood COHb and CN^- levels found in the postmortem samples collected from the victims of fire accidents should be carefully interpreted in view of the potential for the selective presence of these primary combustion gases being absorbed in pooled blood after death.

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