HYDROXYCOBALAMIN AND ASCORBIC ACID MIXTURE (B12r): A POTENTIAL ADJUVANT THERAPY IN THE SETTING OF CARBON MONIXIDE POISONING

VCU Medical Center
Department of Anesthesiology

Roderique J, Guerry C, Somera L, Josef C, Newcomb A, Terner J, Reynolds P, and Spiess B

INTRODUCTION

Carbon monoxide (CO) poisoning is a significant public health burden worldwide. In the US alone, the CDC estimates over 15,000 hospital visits and nearly 500 deaths annually secondary to non-fire related CO exposure.[1] Unlike mortality, long-term cardiovascular and neurologic morbidity is less easily quantified; however, preliminary research raises concerns for both acute high concentration exposures and chronic low concentration exposures.

CO binds human hemoglobin to form carboxyhemoglobin (COHb) with an affinity between 210 and 250 times that of oxygen. Oxygen therapy has been the mainstay of emergent patient management, but patients may still require many hours to clear clinically significant concentrations of CO under normobaric conditions. While the efficacy of hyperbaric oxygen therapy (HBOT) is well established, chamber availability and transport logistics continue to inhibit its effectiveness -- particularly in remote locations or developing health systems.

This study investigates a novel injectable compound, reduced hydroxocobalamin ("$B12_r$"), as a potential therapeutic alternative to HBOT by quantifying its effect on the rate of intravascular conversion of CO to carbon dioxide (CO_2) and the consequent reduction in the half-life ($t_{1/2}$) of COHb.

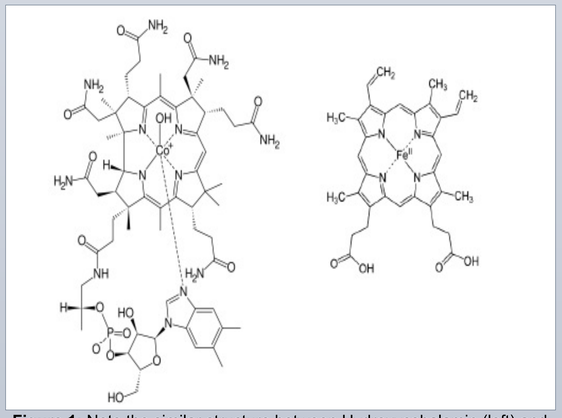

Figure 1: Note the similar structure between Hydroxycobalamin (left) and Heme B (right)

METHODS

The study used a closed-loop artificial circulation system with a hollow-fiber membrane oxygenator circulating fresh whole human blood. The out-flow gas from the oxygenator was monitored in real-time. The reduced form of hydroxocobalamin ("$B12_r$") was generated from a buffered ascorbic acid and B12 mixture and verified using Raman spectroscopy. A COHb concentration of fifty percent was achieved in the circulating blood prior to the injection of a pre-selected concentration of the $B12_r$ solution (Figure 2). Pre- and Post-injection samples were drawn and analyzed by co-oximetry (Radiometer Copenhagen, ABL 825).

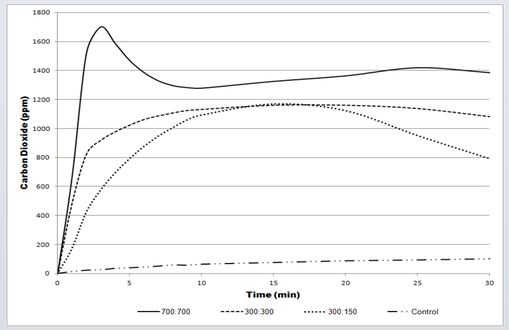

Figure 2: Repeated trials of each dose concentration (n=5/dose): 700 mg OHCbl with 700 mg ascorbic acid; 300 mg OHCbl with 300 mg ascorbic acid; 300 mg OHCbl with 150 mg ascorbic acid. Each line represents the mean response over time and is plotted to show the central tendency of CO_2 generation (in ppm) for each dose mixture over a 30 minute period. The controls (n=5 for each) were: deoxygenated NS (0.9% NaCl solution), 350 mg OHCbl in 5 mL deoxygenated NS, and 350 mg ascorbic acid in 5 mL deoxygenated NS. The control line above represents the aggregate central tendency for all three.

The concentrations of COHb and HbO_2 of the circulating blood were determined in real-time using Raman spectroscopy. Raman spectroscopy was utilized to minimize the interference associated with traditional spectrophotometric techniques (Figure 1).[2] As there is known endogenous production of CO, ^{13}C radiolabeled CO (Cambridge Isotope Laboratories) was used and the $^{13}CO_2/^{12}CO_2$ ratio examined using an infrared spectral analyzer. Adult male Long-Evans rats were exposed to CO and then injected with either normal saline or $B12_r$. Pre- and Post-injection blood samples were analyzed by co-oximetry as above.

RESULTS

$B12_r$ injection resulted in a five-fold increase in the gas-out concentration of CO_2 compared with controls. There was a 16.7% increase in the $^{13}CO_2/^{12}CO_2$ ratio over baseline. Resonance raman analysis demonstrated a reduction in the $t_{1/2}$ of HbCO in the closed-loop system from 43 minutes to 19 minutes (utilizing a 100% oxygenator for clearance). Blood-gas data showed mean pH of 7.29 and pCO2 of 23.9 in placebo rats, versus pH 7.44 and pCO2 31.8 in B12r treated rats.

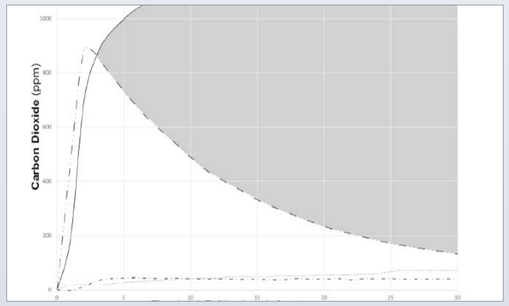

Figure 3: Graph of the median CO2 concentration released by the blood following administration of either the antidote or the components. The shaded area represents the difference between the CO2 produced by the neutralization of ascorbic acid, and that produced by the antidote from CO.

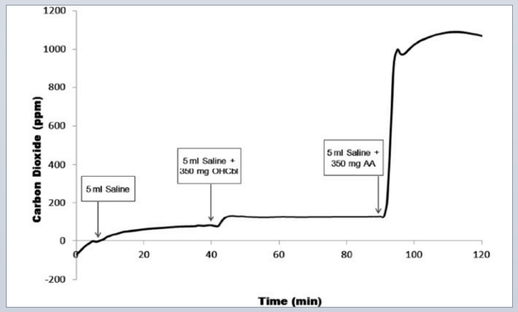

Figure 4: pCO2 after sequential injection of antidote componenets into poisoned blood. The line shows the mean response in CO2 generated (in ppm) over time with sequential injection of 5mL deoxygenated NS, follwed by 350mg OHCbl in 5mL deoxygenated NS, followed by 350mg ascorbic acid in 5mL deoxygenated NS (five samples each).

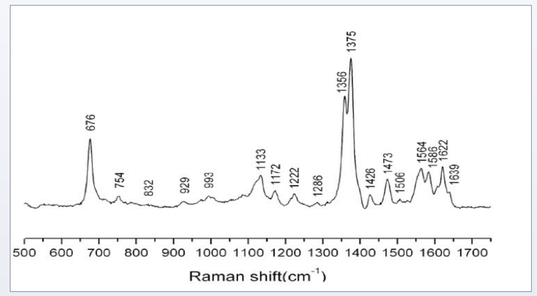

Figure 5: the RR spectrum of whole blood, poisoned with CO (49.2% COHb), immediately prior to antidote injection. The spectrum indicates that the Hgb in the sample is primarily a mixture of deoxyhemoglobin (1356 cm^{-1} and 1472 cm^{-1}) and carboxyhemoglobin (1375 cm^{-1}). The band at 1564 cm^{-1}indicates the presence of methemoglobin.

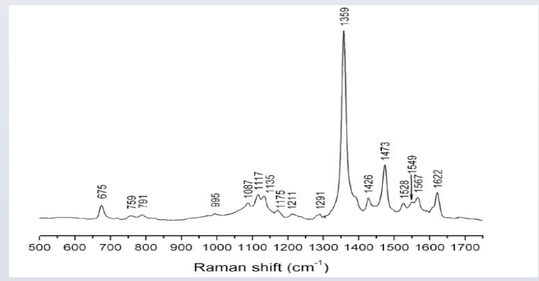

Figure 6: The RR spectrum of the same blood as in figure 5, but 20min after antidote injection. The peaks for the antidote (1602 cm^{-1}, 1537 cm^{-1}, 1570 cm^{-1}, 1496 $cm^{-1}$and 1352 cm^{-1}) are absent – denoting a shift of the antidote into a different resonance enhancement region. Also notable is the presence of only deoxyhemoglobin (1356 cm^{-1} and 1472 cm^{-1}, indicating apparent conversion of the COHb to deoxyhemoglobin.

SUMMARY/CONCLUSIONS

These data indicate that the mixture of hydroxocobalamin and ascorbic acid in blood containing carboxyhemoglobin is capable of converting CO to CO_2. The findings support the potential use of this injectable mixture as an alternative or complement to hyperbaric oxygen therapy in the setting of CO poisoning. Further work is on-going to examine the effect of this antidote on organ O_2 delivery as well as expanding current trials to a large animal model prior to initiating human trials.

REFERENCES AND ACKNOWLEDGEMENTS

1. National Electronic Injury Surveillance System All Injury Program (NEISS-AIP) and National Vital Statistics System (NVSS) cited in CDC MMWR (January 21, 2005 / 54(02); 36-39) Unintentional Non--Fire-Related Carbon Monoxide Exposures
2. Lee J, Mukai D, Kreuter K, Mahon S, Tromberg B, Brenner M. Potential interference by hydroxocobalamin on cooximetry hemoglobin measurements during cyanide and smoke inhalation treatments. Ann Emerg Med 2007;49:802-5

Contact: Christopher Guerry: cguerry@mcvh-vcu.edu

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