

INFLAMMATORY MEDIATORS AND OTHER BIOMARKERS IN CO-INTOXICATED PATIENTS AFTER HYPERBARIC OXYGEN THERAPY (HBO2)

GIULIANO VEZZANI¹, STEPHANIE SOCIAS², ANTONINO BIANCO³, ANTONIO PAOLI⁴, LEONARDO CABERTI¹, LUCA CANTADORI¹, DANIELE MANELLI¹, MARCO MORDACCI¹, DEVANAND MANGAR², ENRICO M. CAMPORESI², GERARDO BOSCO⁴

¹Vaio Hospital, Fidenza (Parma), Italy - ²Florida Gulf-to-Bay Anesthesiology Associates LLC; Tampa General Circle. Tampa, FL, USA - ³Sport and Exercise Sciences Research Unit, University of Palermo, Italy - ⁴Department of Biomedical Sciences, University of Padua, Italy

ABSTRACT

Objective: The present study was conducted in attempt to identify reliable biomarkers in predicting the severity of neurological injury.

Materials and methods: A number of 30 patients with confirmed CO poisoning and a number of 7 healthy control volunteers were involved into the study. All participants received at least 60 minutes of hyperbaric oxygen therapy (HBO2) at 2.5 atmospheres absolute (ATA). Standard blood gas analysis was performed on 10 mL venous blood samples obtained immediately before and after HBO2. Seven biomarkers and carboxyhemoglobin (COHb) levels were measured.

Results: Following HBO2, all patients recovered fully and were discharged in stable condition. Significant differences between pre and post HBO2 values were found in three of the seven biomarkers analyzed: TNF-alpha, IL-10, and S100B. Additionally, S100B and NSE levels were significantly different from controls for the 10 patients who experienced temporary loss of consciousness (LOC), and IL-6 levels were significantly different from controls for all CO-intoxicated patients.

Conclusion: Conclusive evidence of a correlation between a single biomarker and LOC patients was not found. However, the data suggests that the significance of the fall between pre and post HBO2 values for TNF-alpha and IL-10, along with the resolution of IL-6 levels, may herald the severity of the patient's neurological condition.

Key words: Carbon monoxide, Neurological damage, Inflammatory markers, Hyperbaric oxygen.

DOI:10.19193/0393-6384_2016_1_29

Received May 30, 2015; Accepted January 02, 2016

Introduction

Carbon monoxide (CO) is a colorless, odorless yet highly toxic gas. It is a by-product of the incomplete combustion of hydrocarbons commonly found in engine exhaust and heating units that burn oils, coal, and kerosene⁽¹⁾. CO intoxication is one of the leading causes of poison related injury in the modern world⁽²⁾. Although small amounts of CO are needed for normal physiological functioning, exogenous exposure is toxic⁽³⁾. CO induces toxicity through hypoxic and inflammatory pathways⁽⁴⁾. CO quickly binds hemoglobin to create carboxyhemoglobin and deprive tissues of oxygen⁽³⁾. With high demands for oxygen, the brain and heart are most

susceptible to injury⁽⁴⁾. Inflammation occurs with a more gradual progression due to the oxidative stress brought on by increased levels of cytosolic heme and heme oxygenase-1. Although inflammation occurs independent of hypoxia, it has also been invoked as a cause for cardiac and neurological injury. Yet, the level of carbon monoxide needed to cause inflammation is uncertain⁽³⁾. Hyperbaric oxygen therapy (HBO2) is the primary method of treatment for CO poisoning and for local tissue anoxia, and its urgent application is universally accepted^(3,5-7). Although elevated carboxyhemoglobin levels indicate previous exposure to CO, the level does not correlate with clinical symptoms or long-term prognosis⁽⁴⁾. CO poisoning involves a wide array of

symptoms that may be attributed to various other conditions. This high variability in signs and clinical symptoms of CO poisoning, combined with a lack of specific diagnostic tools, makes it very difficult to identify the severity and further progression of both cardiac and neurological injury. While previous studies have identified reliable biomarkers for predicting cardiac injury, namely troponin-T and N-terminal pro-brain natriuretic peptide (NT-pro BNP), analogous biomarkers for brain injury have not been found⁽⁸⁾. A number of reports have described increases in neuron specific enolase (NSE) and S100B proteins as possible indicators of neurological damage, but the results have been inconclusive^(1,9-11). The absence of a correlation between carboxyhemoglobin levels and severity of the clinical outcome suggests that other processes such as inflammation, rather than hypoxia, may be indicative of final evolution and later outcomes⁽³⁾. We therefore analyzed the levels of inflammatory and brain specific markers in a recent series of CO-intoxicated patients and healthy volunteers, to identify a possible correlation between biomarkers and adverse neurological sequelae. We hypothesized that elevations in specific biomarkers and significant reductions after hyperbaric treatment may correlate with neurological injury severity.

Materials and methods

The public hyperbaric facility at the Vaio Hospital, near Parma, Italy, received and treated all participants (CO-intoxicated patients transferred in from up to 200 miles away). The standard protocol approved by the Ethics Committee at Vaio Hospital mandates the collection of 10 mL venous blood samples immediately before and after HBO2 treatment for adult patients of suspected CO poisoning to evaluate the severity of intoxication and to guide clinical treatment. This standard protocol was presented to the Vaio Hospital Ethics Committee and deemed exempt from acquiring individual patient consent due to the urgency of treatment, possible unconsciousness of subjects, and the small quantity of blood collected. Abnormal results have been disclosed to all patients. The study protocol also addressed the potential utility of obtaining HBO2 blood samples from healthy volunteers, namely the physicians and nurses accompanying the patient inside the multiplace hyperbaric chamber for assistance. These clinicians all gave written, informed consent to the study.

In this study, 30 patients admitted from 10/2013 to 12/2013 were evaluated. Following protocol, blood samples were taken immediately before and after HBO2. As some patients were transferred to the Hospital from outside Emergency Room facilities, the COHb levels obtained at those initial facilities of presentation were also used in our final calculations.

HBO2 was initiated urgently, within a few hours of the CO exposure, and consisted of 2.5 ATA O2 for 60 minutes, including compression and decompression times. The 10 patients who had been rescued with loss of consciousness (LOC) received an additional 30 minutes of HBO2 at 2.8 ATA before the 2.5 ATA treatments.

Study population

Our analysis excluded patients who were admitted comatose and/or intubated, as these patients often present with longer delays and require more complex management. Identical measures were obtained from all 30 patients with confirmed CO poisoning and from the 7 healthy, asymptomatic control volunteers. All patients and volunteers received 60min of HBO2 at 2.5 ATA. The 30 patients were divided into two groups: loss of consciousness (LOC) and no loss of consciousness (No LOC). Patients in the LOC group experienced transient loss of consciousness immediately after CO exposure, but all regained consciousness by the time HBO2 was initiated; whereas the patients in the No LOC group remained conscious from the time of exposure to treatment. All patients, therefore, were conscious at the time of initiating HBO2. Beyond the standard 60 minutes of HBO2 at 2.5 ATA given to all patients, LOC patients received an initial additional 30 minutes of HBO2 at 2.8 ATA. Patients were evenly split male-female, and there were no differences in mean age between LOC and No LOC patients nor in starting COHb values, which are reported here from the initial admission data from peripheral hospitals (Table 1). The seven healthy volunteers were all adult operational support personnel, either physicians or nurses, familiar with HBO2 exposure. Venous blood samples were obtained immediately before and after HBO2 and analyzed via ELISA Kit. Additional measured data included: interleukin (IL)-6, IL-8, IL-10, C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), neuron-specific enolase (NSE), and S100B protein. COHb levels upon first presentation were also reported from blood samples taken at the

Emergency Rooms of original. Data Collection and Measurements presentation and, when available, are shown in Table 1.

| Table 1. Patient Characteristics | | | | | |
|----------------------------------|-------------|----------|-------------------|-----------------|------------------|
| | Male/Female | Mean Age | Time to HBO2 (hr) | Pre % COHb Mean | Post % COHb Mean |
| All Patients# | 15/15 | 42.6 | | 24.30 | 5.1* |
| LOC | 5/5 | 41.8 | 6.0 | 26.8 | 3.8* |
| No LOC | 10/10 | 43.1 | 4.7 | 23.1 | 5.8* |

#All patients = LOC + No LOC, does not include controls; *Values are significantly different from pre HBO2 values.

Patient outcome and follow-up

All patients improved and were asymptomatic after receiving a single round of hyperbaric treatment. Neurologic evaluations were performed at arrival and daily until discharge, if admitted to the Hospital. Follow-up telephone interviews were completed for all patients at 10, 20, and 30 days post HBO2.

Data analysis

We hypothesized that elevations in specific biomarkers may correlate with adverse neurological sequelae. Maximum differences in pre and post HBO2 biomarker values were expected in the most severely poisoned patients, the LOC group. We tested if the levels of certain biomarkers could be used to identify the patients most at-risk to develop late sequelae. Statistical analysis was performed using SPSS statistical software vers. 18 (SPSS, Inc., Chicago). Pre and post HBO2 biomarker values from all patient groups were compared against controls using unpaired t-tests with significance set at 0.05. Pre vs post HBO2 biomarker values were compared within patient groups using Wilcoxon two-tailed signed-ranks tests with significance set at 0.05.

Results

Following HBO2, all patients recovered fully and were discharged from the hospital in stable condition. Neurologic symptoms were observed at the beginning of HBO2 treatment in 5 patients: 4 reported confusion and malaise and 1 complained of a headache.

All neurologic symptoms resolved after 1 HBO2 without long-term sequelae on follow-up by telephone interviews 10, 20, and 30 days after HBO2. Significant differences between pre and post HBO2 values were found in three of the seven biomarkers analyzed: TNF-alpha values (Fig. 1)

were significantly different for the All combined patient group and for the No LOC patient group; IL-10 values (Fig. 2) were significantly different for the All patient group only; and S100B values were significantly different for all three groups (Fig. 3). These significant pre and post HBO2 differences found in TNF-alpha, IL-10, and S100B were not observed in the controls.

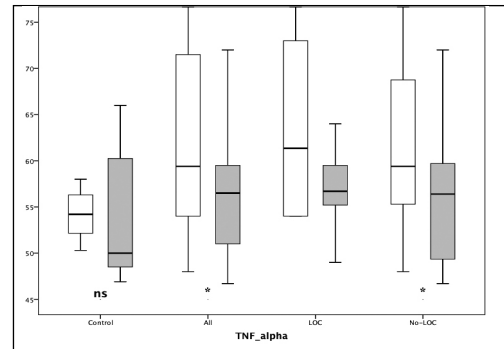


Fig. 1: TNF-α pre and post-HBO2 values for controls and patient groups. *Values are significant.

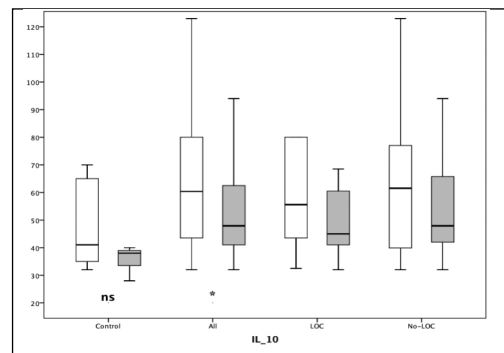


Fig. 2: IL-10 pre and post-HBO2 values for controls and patient groups. *Values are significant.

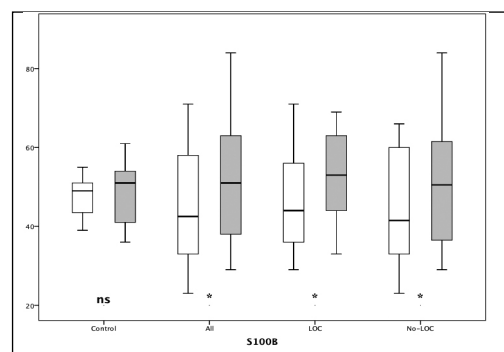


Fig. 3: S100B pre and post-HBO2 values for controls and patient groups. *Values are significant.

Tables 2 and 3 display mean values and significance for all biomarkers. All three patient groups exhibited a significant decrease in COHb levels after HBO2 (Table 1). LOC patients had slightly higher COHb presenting values, but not significantly different from patients without LOC.

| | S100B | | Neuron Specific Enolase | | C Reactive Protein | |
|--|-----------|------------|-------------------------|----------|--------------------|-----------|
| | PreHBO2 | PostHBO2 | PreHBO2 | PostHBO2 | PreHBO2 | PostHBO2 |
| Controls | 47.4±6.1 | 48.29±9.0 | 2.0±0.2 | 2.4±0.4* | 0.07±0.03 | 0.08±0.03 |
| All Patients (not including controls) | 45.4±14.3 | 50.7±14.0* | 3.3±2.5 | 3.2±1.3 | 0.14±0.13 | 0.16±0.17 |
| LOC Patients | 46.5±13.2 | 52.4±12.1* | 2.8±0.7 | 3.4±1.5 | 0.09±0.09 | 0.09±0.09 |
| No-LOC Patients | 44.8±15.1 | 49.9±15.0* | 3.6±3.0 | 3.1±1.2 | 0.17±0.14 | 0.19±0.18 |

Values are mean ± SD. *Values are significantly different from pre HBO2 values.

| | IL-6 | | IL-8 | | IL-10 | | TNF-α | |
|--|-----------|------------|-------------|-------------|-----------|------------|-----------|------------|
| | PreHBO2 | PostHBO2 | PreHBO2 | PostHBO2 | PreHBO2 | PostHBO2 | PreHBO2 | PostHBO2 |
| Controls | 9.6±0.8 | 10.8±1.5 | 358.8±253.5 | 97.9±51.6* | 56.1±31.6 | 38.7±10.2 | 56.0±12.9 | 54.3±7.7 |
| All Patients (not including controls) | 41.2±37.5 | 58.2±97.3 | 258.8±376.3 | 306.0±590.8 | 70.8±54.6 | 51.9±15.2* | 67.8±30.6 | 62.7±27.7* |
| LOC Patients | 47.1±43.5 | 67.4±124.4 | 312.0±453.5 | 371.2±748.9 | 89.4±88.5 | 48.8±12.6 | 66.0±14.0 | 56.9±3.9 |
| No-LOC Patients | 38.3±35.0 | 53.6±84.0 | 232.1±341.2 | 273.4±513.6 | 61.5±23.8 | 53.5±16.4 | 68.7±36.5 | 58.1±12.4* |

Values are mean ± SD. *Values are significantly different from preHBO2 values.

Mean COHb levels for LOC and No LOC patient groups were 26.8% and 23.1%, respectively. In controls, low COHb levels without significant changes were found. Aside from pre and post HBO2 value differences, concentrations of three biomarkers were significantly different from controls for at least one patient group: S100B and NSE were significantly different from controls for the LOC patient group and IL-6 was significantly different from controls for all three patient groups (All, LOC, and No-LOC).

Discussion

CO exposure causes a release of various proteins into the bloodstream via hypoxic and inflammatory pathways, either of which may herald neurological damage. Previous toxicology data from CO poisoning patients has shown that acute inflammatory modulation may mediate such neurological toxicity^(8, 12). Our study thus analyzed proteins with suspected involvement in both pathways. TNF-alpha, IL-6, IL-8, and IL-10 are inflammatory mediators, while S100B and NSE are neural tissue proteins characteristic of hypoxic injury⁽¹²⁾.

Previous studies have suggested that S100B and NSE, proteins released from neuronal and astroglial cells following hypoxic brain injury, may be indicative of neurological deficit. However, the evidence is inconclusive. Brvar et al. found elevated S100B levels in unconscious patients exposed to CO for a long period of time, but only one of those patients had any neurological sequelae at discharge. No S100B elevation was found in patients who experienced only transitory unconsciousness⁽⁹⁾.

Rasmussen et al. found no changes in either S100B or NSE levels after CO poisoning⁽¹¹⁾. Both Cakir et al. and Yordan et al. noted increased S100B

levels but not NSE levels. However, only Cakir reported considerable decreases in NSE and S100B after HBO2. Yordan found no difference in either S100B or NSE levels after HBO2^(1, 12, 13). Our results add further ambiguity to the role of S100B and NSE in predicting neurological injury. In regards to S100B, we found no difference when compared to controls. NSE levels were significantly different from controls in the LOC patient group only. In comparing pre and post HBO2 values, however, all patient groups experienced a significant change in S100B while no patient groups experienced a significant change in NSE. Furthermore, our S100B data differs from all other reports in that our patients presented with initially low S100B levels that subsequently rose following HBO2, rather than initially high levels that later fell. In addition to our analysis of S100B and NSE as suspected products of hypoxic injury, we also evaluated the levels of various inflammatory mediators, particularly TNF-alpha, IL-6, IL-8, and IL-10. We hypothesized that these proteins, which are involved in the inflammatory rather than hypoxic path of CO intoxication, might be more telling of neurological injury.

Our results indicate that a rapid decrease in the inflammatory proteins TNF-alpha and IL-10 after HBO2 may predict the resolution of neurologic symptoms; while protein levels that remain elevated after HBO2 may be indicative of neurological complications. We propose further study of TNF-alpha and IL-10 monitoring in CO poisoning patients as a marker of neurological complications, especially if residual symptoms persist after HBO2.

Limitations

There are several limitations to our study. We only analyzed 30 patients (10 LOC patients and 20 No-LOC patients). While this small sample size

provides a basis for identifying significant differences in biomarkers, it is not a fully exhaustive approach. To fully identify all biomarker levels with significant differences between pre and post-HBO2 values, future studies are needed. For example, in our LOC patient group, pre and post-HBO2 TNF- α value differences approached significance, but, with a p-value of 0.06, did not meet the 0.05 significance level. Additionally, as all patients experienced only mild CO intoxication and none experienced major neurological symptoms, the homogeneity of the study population suggests that our results do not constitute a proof but rather provide a strong suspicion of which biomarkers might herald neurological sequelae. Finally, the delay in taking blood samples might also influence results. Although COHb levels were taken at the ER of original presentation, blood samples for biomarker analysis were not taken until right before HBO2 treatment. As plasma biomarker levels vary greatly over time, temporal adjustments to blood collection could offer better discrimination of patient groups. A prospective study involving the use of objective neuropsychological testing is planned to more accurately assess whether our evidenced variations in plasma proteins provide the objective method for stratifying CO poisoning we suspect.

Conclusion

Overall, we did not produce conclusive evidence of a correlation between a single biomarker and neurological complications. However, we found that two inflammatory mediators, IL-10 and TNF- α , decreased significantly for the All patient group following HBO2. Although statistically unclear due to our small sample size, this difference is likely greater for LOC patients. Additionally, we found that IL-6 was significantly elevated in all three patient groups when compared to controls and that after HBO2, these IL-6 levels dropped to levels very similar to that of controls. Our data suggests that the significance of the fall between pre and post HBO2 values for TNF- α and IL-10, along with the resolution of IL-6 levels, may herald the gravity of the patient's neurological condition. We propose further investigation into TNF- α , IL-10, and IL-6 concentrations in CO-intoxicated patients to better evaluate the efficacy of these biomarkers as indicators of neurological complications.

References

- 1) Akelma AZ, Celik A, Ozdemir O, Akelma FK, Abaci A, Razi CH, Kislal FM, Akin O. *Neuron-specific enolase and S100B protein in children with carbon monoxide poisoning: children are not just small adults*. Am J of Emergency Med 2013; 31: 524-528.
- 2) Prockop L, Chichkova R. *Carbon monoxide intoxication: An updated review*. J of the Neurological Sciences 2007; 262: 122-130.
- 3) Weaver LK. *Carbon Monoxide Poisoning*. N Engl J Med 2009; 360: 1217-25.
- 4) Lippi G, Rastelli G, Meschi T, Borghi L, Cervellin G. *Pathophysiology, clinics, diagnosis and treatments of heart involvement in carbon monoxide poisoning*. Clin Biochem 2012; 45: 1278-85.
- 5) Martines F, Dispenza F, Gagliardo C, Martines E, Bentivegna D. *Sudden sensorineural hearing loss as prodromal symptom of anterior inferior cerebellar artery infarction*. ORL J Otorhinolaryngol Relat Spec. 2011; 73(3): 137-40. doi: 10.1159/000327523.
- 6) Rabczyński M, Kuźnik E, Guziński M, Adamiec R. *Critical ischemia of the fingers in an auto mechanic as a result of occupational exposure*. Int J Occup Med Environ Health. 2014 Sep 10. [Epub ahead of print].
- 7) Martines F, Sireci F, Cannizzaro E, Costanzo R, Martines E, Mucia M, Plescia F, Salvago P. *Clinical observations and risk factors for tinnitus in a Sicilian cohort*. Eur Arch Otorhinolaryngol. 2015 Oct; 272(10): 2719-29. doi: 10.1007/s00405-014-3275-0.
- 8) Thom SR, Bhopale VM, Milovanova TM, Hardy KR, Logue CJ, Lambert DS, Troxel AB, Ballard K, Eisinger D. *Plasma biomarkers in carbon monoxide poisoning*. Clin Toxicol 2010; 48: 47-56.
- 9) Brvar M, Mozina H, Osredkar J, Mozina M, Bručan A, Bunc M. *The potential value of the protein S-B level as a criterion for hyperbaric oxygen treatment and prognostic marker in carbon monoxide poisoned patients*. Resuscitation 2003; 56: 105-109.
- 10) Ide T, Kamijo Y, Ide A, Yoshimura K, Nishikawa T, Soma K, Mochizuki H. *Elevated S100B level in cerebrospinal fluid could predict poor outcome of carbon monoxide poisoning*. Am J of Emergency Med 2012; 30: 222-225.
- 11) Rasmussen LS, Poulsen MG, Christiansen M, Jansen EC. *Biochemical markers for brain damage after carbon monoxide poisoning*. Acta Anaesthesiol Scand 2004; 48(4): 469-73.
- 12) Cakir Z, Aslan S, Umudum Z, Acemoglu H, Akoz A, Turkyilmaz S, Ozturk N. *S-100B and neuron-specific enolase levels in carbon monoxide-related brain injury*. Am J Emerg Med 2010; 28: 61-67.
- 13) Yardan T, Cevik Y, Donderici O, Kavalci C, Yilmaz FM, Vural K, Yuzbasioglu Y, Gunaydin YK, Sezer AA. *Elevated serum S100B protein and neuron specific enolase levels in carbon monoxide poisoning*. Am J Emerg Med 2009; 27(7): 838-42.

Corresponding author

ANTONINO BIANCO, PhD

Assistant Professor

Sport and Exercise Sciences Research Unit

University of Palermo

Via Giovanni Pascoli 6, 90144 Palermo, (Italy)